MEETING

SCIENTIFIC ADVISORY BOARD

MARCH 14, 15, 16, 1973

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H. Wakeham

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

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H Wakeham
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Mar 14, 1973

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AGENDA

SCIENTIFIC ADVISORY BOARD

March 14-15-16, 1973

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1. Interim actions - to be considered or confirmed

    a. Administrative

    b. Planning Committee

2. Applications

    Book I - Renewals

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THE COUNCIL FOR TOBACCO RESEARCH – U.S.A., INC.

CONFIDENTIAL REPORT

SCIENTIFIC ADVISORY BOARD MEETING

NEW YORK, NEW YORK

SEPTEMBER 15-17, 1972

Dr. Sheldon C. Sommers, Chairman (9/15,17)	
Dr. Howard B. Andervont	
Dr. Richard J. Bing	
Dr. McKeen Cattell	
Dr. William U. Gardner	Scientific Consultant, CTR
Dr. Robert J. Huebner	
Dr. Leon O. Jacobson	
Dr. Kenneth M. Lynch	
Dr. Hans Meier	
Dr. Murray Senkus	Chairman, ITC
Dr. Alvan R. Feinstein (9/17)	
Dr. Carl C. Seltzer (9/17)	
Dr. John P. Wyatt (9/17)	
Mr. H. H. Ramm	President, CTR
Mr. W. T. Hoyt	Executive Vice President, CTR
Dr. Robert C. Hockett	Acting Scientific Director, CTR
Dr. J. Morrison Brady	Associate Scientific Director, CTR
Dr. John H. Kreisher	Associate Scientific Director, CTR
Dr. Frederic W. Nordsiek	Associate Scientific Director, CTR
Dr. Vincent F. Lisanti	Scientific Associate, CTR
Mr. Leonard S. Zahn	Consultant, CTR

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1. The report of actions taken at the June meeting was approved.

2. The following administrative actions were approved:

413-AR3 Cecile Leuchtenberger, Ph.D. A time extension without additional funds was approved for the period January 1 to April 30, 1973.

467-A Thomas C. Westfall, Ph.D. Permission was granted to transfer $210.35 from Supplies to Travel.

547-C Joseph J. Guarneri, Ph.D. Permission was granted to transfer $1,290.00 from Salaries to Equipment.

572-BR1S T. Timothy Crocker, M.D. Expenses and per diem in the amount of $838.50 were approved for Dr. Jay A. Levy for the months of May, June and July.

Source: https://www.industrydocumentslibrary.ucsf.edu/tobacco/yly10000

573-BS Clayton G. Loosli, M.D., Ph.D. This supplement covers the amount of $338.29 (including tax and shipping) paid to Process & Instruments Corporation for one pair of large mouse animal cone holders with stirer assembly mounted on one of the pair.

603-AR2 Shirley L. Kauffman, M.D. An unexpended balance of $638.80 was returned and deposited.

758-B Hans Meier, D.V.M. Permission was granted to transfer $1,490.00 from Salaries to Equipment.

763 John W. Parker, M.D. Permission was granted to transfer the unexpended balance of $329.63 to 763R1.

776M2 Elliott S. Vessell, M.D. Permission was granted to transfer the unexpended balance of $807.29 to 776M2R1.

800 J. A. Hayes, M.D. Permission was granted to extend this grant from July 1 to September 30, 1972 and then from October 1 to December 31, 1972 without additional funds.

814 Una Smith, Ph.D. Permission was granted to extend the grant from October 1, 1972 to January 31, 1973, using the unexpended balance during this period for purposes of the project as well as to improve electron microscopy space in new quarters.

815 John R. Esterly, M.D. An extension of the grant was authorized from July 1 to September 30, 1972, and permission was granted to use the unexpended balance of $6,000.00 during this period.

836 Walter B. Essman, Ph.D. Permission was granted to transfer funds from Salaries to three other categories.

841 Charles Mittman, M.D. The sum finally approved by the staff is $35,000.00.

Contract 4-BS Bio-Research Institute, Incorporated. $1,900.00 Approved for Kentucky reference cigarettes and UPS charges.

7 University of Southern California. An unexpended balance of $12,973.19 was returned and deposited.

9-BS University of San Francisco. Expenses and per diem in the amount of $224.00 for the period April 1 to June 30, 1972 were approved for Dr. Robert M. Kovatch.

12 Lorillard Smoking Machine. On page 5 of the June 1972 minutes, an authorization of $1,500.00 was reported for production of cone holders. The final amount totaled $1,277.40.

3. Dr. Sommers reported on the Planning Committee meeting held August 26, 1972 in New York City. Policy recommendations and interim actions taken by the Committee are contained in this Scientific Advisory Board meeting report.

Source: https://www.industrydocuments.ucsf.edu/docs/yn0000

4. Upon the recommendation of the Planning Committee, it was decided to hold two Scientific Advisory Board meetings a year instead of four. These will be full three-day meetings and will be held either Wednesday-Friday or Thursday-Saturday inclusive during March or April and September or October. The next meeting of the Board was therefore scheduled for March 14-15-16, 1973.

5. Upon recommendation of the Planning Committee a new method of SAB voting procedure at meetings was approved. After the usual discussion and vote to approve or disapprove are undertaken, there will be an anonymous ballot on each application to give an overall rating of 1 to 5, 1 being highest. After grants are rated the staff will average the vote and make a determination as to dollar awards based on competitive scoring and the funds available.

6. Scientific Advisory Board emeritus membership was discussed. Dr. Rienhoff has already asked for emeritus status. It was considered necessary to add certain specialist members to the Board without greatly enlarging it. It is hoped that emeritus members will remain available for consultation and attend some meetings.

7. Recognizing that the next SAB meeting is not scheduled until March:

(a) The staff was authorized to approve the renewal of one grant and one contract anticipated on January 1, 1973, as follows:

833R1 A. Stanley Weltman, Ph.D. $27,663.00

2E Microbiological Associates, Inc. $157,000.00. This includes $7,000.00 for the maintenance of the Snell mouse colony.

(b) The Planning Committee was authorized to take such other interim actions as it felt proper before the March SAB meeting.

8. Renewals

573-BR1 Clayton G. Loosli, M.D. $105,989.00 Approved for the second year of an originally proposed three-year project.

843R1 A. C. Barger, M.D. $57,200.00 Approved for $50,000.00 for the second year of an originally proposed three-year project.

9. New Applications

702-A A. H. Niden, M.D. $25,618.00 Approved for one year of a proposed three-year project.

725-C Jack Chalon, M.D. $18,110.00 Deferred.

800 J. A. Hayes, M.D. $48,017.00 Denied.

833S A. S. Weltman, Ph.D. $15,000.00 Approved subject to a site visit by the Board Chairman.

836-A Walter B. Essman, Ph.D. $53,407.00 Approved for $40,000.00 for one year of a proposed three-year project. An additional 3 months pro rata of $10,000.00 was approved to make the anniversary date January 1, 1974, making this award equal to a total of $50,000.00.

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Source: https://www.industrydocuments.ucsf.edu/docs/yyfl0000

864 T. A. Slotkin, Ph.D. $12,109.00 Approved for one year of a proposed two-year project.

865 R. H. Carlson, Ph.D. $6,981.00 Denied.

866 Herschel Sandberg, M.D. $15,567.00 Denied.

867 Abraham S. Feigenbaum, Ph.D. $19,012.00 Denied.

868 Herbert A. McKennis, Ph.D. $68,300.00 Approved for $60,000.00 for one year of a proposed three-year project.

869 Ronald P. Rubin, Ph.D. $21,300.00 Approved for one year of a proposed three-year project.

871 Ines Mandl, Ph.D. $39,882.00 Denied.

872 Donald J. Massaro, M.D. $46,400.00 Denied.

873 Edward F. Domino, M.D. $42,157.00 Denied.

874 H. G. Petering, Ph.D. $40,562.00 Denied.

875 Sheldon C. Sommers, M.D. $90,474.00 Approved for one year of a proposed three-year project.

876 Henry Yeager, M.D. $13,960.00 Denied.

877 Herbert B. Herscowitz, Ph.D. $32,623.00 Approved for one year of a proposed three-year project.

878 Paul Hamosh, M.D. $26,870.00 Approved for one year of a proposed three-year project.

10. Smoking Machines

Following an extensive discussion of smoking machines with special reference to the Walton and Lorillard machines, it was the opinion of the Board that testing procedures as outlined were important and should be done. The Industry Technical Committee having previously given its advice as to the competence of Oak Ridge to perform the work, the Board requested the Planning Committee to arrange for the drafting of a contract, along the lines of the Oak Ridge proposal excluding the cytology section and gave its approval to such contract provided it was satisfactory to the Planning Committee and funds were available for the purpose.

11. Contracts

Status reports on each outstanding contract were given by the contract monitors.

12. The Board approved the proposed Macrophage Conference scheduled for the last week in November.

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Source: https://www.industrydocuments.ucsf.edu/docs/tyl0000

13. Attending the meeting on September 17, 1972 were the following guests:

Alvan Feinstein, M.D., Yale University School of Medicine
Carl C. Seltzer, Ph.D., Harvard University School of Public Health
John P. Wyatt, M.D., University of Manitoba

Drs. Feinstein and Seltzer discussed Epidemiology at length, and Dr. Wyatt made a presentation on the subject of Emphysema.

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INTERIM ACTIONS

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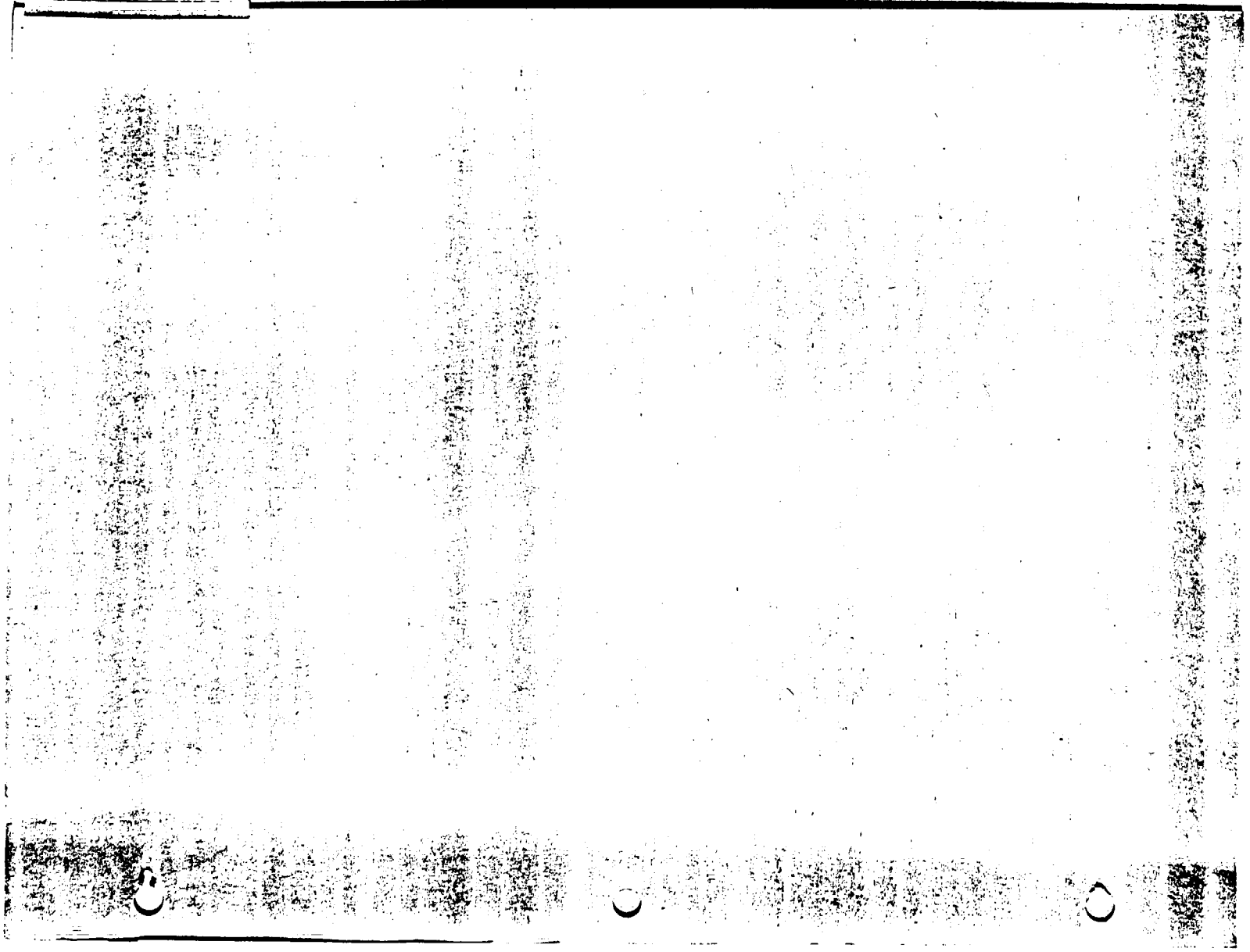

Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

ADMINISTRATIVE REPORT

467-B Thomas C. Westfall, Ph.D. Permission was granted to extend this grant from December 31, 1972 to March 31, 1973 with no additional funds.

519-BS Louis A. Soloff, M.D. A supplementary grant was approved in the amount of $5,000.00 to provide additional funds for purchase of consumable supplies and certain permanent equipment vital to the current project.

547-C Joseph J. Guarneri, Ph.D. Permission was granted to carry forward the unexpended balance of $549.59 to 547-CR1.

548-A S. N. Pradhan, M.D., Ph.D. Permission was granted to extend this grant for a second time from December 31, 1972 to September 30, 1973 with no additional funds.

623-BR1 Walter B. Essman, Ph.D. Permission was granted (1) to use unexpended funds in the approximate amount of $4,190.77, and (2) to extend the project from January 31, 1973 to March 31, 1973.

646-AR1 Robert C. Rosan, M.D. Permission was granted to transfer $4,900.00 from Salaries to Equipment for the purchase of an electrophoresis scanner.

702R2 Albert H. Niden, M.D. An unexpended balance of $204.51 was received and deposited.

704 Lucile Smith, Ph.D. An unexpended balance of $1,321.50 was received and deposited.

734R2 Carroll E. Cross, M.D. Permission was granted to transfer $1,200.00 from Salaries to Supplies, and $1,800.00 from Salaries to Equipment. Since overhead is not allowed for Equipment, a check for $270.00 (representing 15% of $1,800.00) was returned by the University authorities and deposited. The grant therefore has been reduced from $23,175.00 to $22,905.00.

764R1 Charles G. Cochrane, M.D. Permission was granted to carry forward the unexpended balance of $644.30, thus reducing 764R2 from $31,269.00 to $30,624.70.

814 Una Smith, Ph.D. The September minutes reported a time extension without additional funds from September 30, 1972 to January 31, 1973. This has been amended from September 30 to December 31, 1972. The renewal grant (814R1) therefore became effective on January 1, 1973.

826 Thomas R. Dawber, M.D. Permission was granted to carry forward the unexpended balance of $11,324.64 to the current grant 826R1.

833 A. Stanley Weltman, Ph.D. An unexpended balance of $3,665.71 was received and deposited.

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Source: https://www.industrydocuments.ucsf.edu/docs/ytj0000

839 Edwin R. Fisher, M.D. Permission was granted to carry forward the unexpended balance of $6,246.00 to the current grant 839R1.

854 Miss Eleanor J. Macdonald Permission was granted to carry forward the unexpended balance of $794.31 for the purposes of the project.

864 T. A. Slotkin, Ph.D. The September minutes reported the amount approved as $12,109.00. The correct amount approved was $12,110.00.

869 Ronald P. Rubin, Ph.D. Permission was granted to reallocate $2,425.00 from Equipment to Salaries.

891 Dr. Georg B. Neurath The December 1972 Planning Committee meeting minutes reported the amount approved as $41,007.50. The correct amount approved was $41,000.00.

Contract 1-C Mason Research Institute The December 1971 minutes approved an appropriation in the amount of $7,000.00 and in the March 1972 minutes $15,000.00 to defray the salary and expenses of Dr. Charles W. Nystrom of R. J. Reynolds Tobacco Company. These amounted in full to $13,437.19, and the balance remaining of $8,562.81 is cancelled.

2-D Microbiological Associates, Inc. An extension from February 1 to March 31, 1973 was approved on a pro rata basis not to exceed a total of $25,000.00 during that period.

3-B IIT Research Institute The following per diem and expenses were paid Dr. G. P. Brierly for consultation and work on the contract: Per Diem $240.00 and expenses $165.00, totaling $405.00.

9-B University of San Francisco Expenses and per diem in the amount of $442.50 for the period July 1 to September 30, 1972 were approved for Dr. Robert M. Kovatch, and $624.50 for the period October 1 to December 31, 1972.

Expenses and per diem in the amount of $417.15 for Dr. Jay A. Levy were approved.

1003539115

Source: https://www.industrydocuments.ucsf.edu/docs/rjyb0000

THE COUNCIL FOR TOBACCO RESEARCH–U.S.A., INC.

REPORT

PLANNING COMMITTEE MEETING

FRIDAY, DECEMBER 8, 1972

ATTENDANCE

Sheldon C. Sommers, Chairman
Leon O. Jacobson
Clayton G. Loosli

William U. Gardner
Robert C. Hockett
Frederic W. Nordsiek

H. H. Ramm
W. T. Hoyt

1. The report of the August 26, 1972 meeting of the Planning Committee, which had been submitted to and accepted by the Scientific Advisory Board at its September meeting, was approved.

2. The Chairman noted that the Scientific Advisory Board had authorized the Planning Committee to take such interim actions as it felt proper before the March 1973 Board meeting.

3. Renewal Applications

833R1 A. Stanley Weltman, Ph.D. $54,895.00 Approved for a total of $42,663.00, which figure includes $11,250.00 of the supplement approved at the September Scientific Advisory Board meeting.

Contract 2-E Microbiological Associates Incorporated. $157,000.00 Approved with the understanding that the staff is to make a site visit before the anniversary date of the contract and arrange for satisfactory protocols before a final authorization is given.

4. New Applications

588-D B. Bhagat, Ph.D. $36,805.00 Approved for a six-month pro rata amount equal to $18,403.00. Final action is to be determined at the March Board meeting.

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Source: https://www.industrydocuments.ucsf.edu/docs/yyyl0000

572-BR1S Jay A. Levy, M.D. $31,478.00 Approved for $20,000.00 covering the period through March 31, 1973. Any further consideration will depend on action taken by the Scientific Advisory Board.

642-B Leonide Goldstein, D.Sc. $28,579.00 Approved for three months on a pro rata basis in the amount of $7,145.00 for final determination at the March Scientific Advisory Board meeting. If the Board denies the application, a second three-month pro rata payment is to be made.

741-A Joseph M. Lauweryns, M.D. $38,806.00 Approved for three months on a pro rata basis in the amount of $5,273.00 (without allowance for the scanning electron microscope) for final determination at the March Scientific Advisory Board meeting. If the Board denies the application, a second three-month pro rata payment is to be made.

787-A Gary D. Friedman, M.D. $101,100.00 Approved for three months on a pro rata basis in the amount of $25,275.00 for final determination at the March Scientific Advisory Board meeting.

870 H. J. Eysenck, Ph.D. $21,000.00 Postponed for consideration by the Scientific Advisory Board in March 1973.

885 Carl C. Seltzer, Ph.D. $21,250.00 Approved for one year of a proposed two-year project.

889 Timothy J. Regan, M.D. $44,776.00 Approved for one year of a proposed three-year project.

891 Dr. Georg B. Neurath $41,007.50 Approved for one year of a proposed three-year project.

892 Lars Friberg, M.D. $40,095.00 Approved as the total appropriation covering a period of two years.

5. Dr. Rune Cederlöf's request for up to $7,000.00 additional funds to assist his preliminary efforts to arrange for a twin conference was approved as a staff service when and if required.

6. Thirteen cases of investigators who made informal inquiries were reviewed. Six were encouraged to submit applications, five were discouraged, and two were deferred pending site visits to obtain more information.

7. The need for a continuing source of supply of reference cigarettes was recognized. The Planning Committee authorized up to $100,000.00 from the 1973 budget, to be allocated by the staff when and as approved, for the purchase of reference cigarettes as needed by Council grantees and contractors.

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Source: https://www.industrydocuments.ucsf.edu/docs/jgjf0000

8. Miscellaneous

702R2 Albert H. Niden, M.D. A grant award of $25,618.00 effective October 1, 1972 was reduced to $8,240.00, the amount required to retain personnel working on the project during the period when Dr. Niden is making a decision whether or not to change his affiliation.

826 Thomas R. Dawber, M.D. Permission was granted to carry over $11,000.00 remaining on hand at June 30, 1972 to the current grant.

839 Edwin R. Fisher, M.D. Permission was granted to carry over $6,246.00 remaining on hand at September 30, 1972 to the current grant.

- A request for an appropriation of $62,500.00 to produce Walton Horizontal Smoke Exposure machines that will replace outstanding old machines was turned over for a staff decision under the 1973 budget.

9. The suggestion that henceforth planning activities should be a function of the staff was approved. It was also recognized that with the Scientific Advisory Board holding meetings twice a year there would be need for interim decisions; therefore, it was decided to turn the Planning Committee into an Executive Committee of the Scientific Advisory Board, the Chairman of the Board to be the Chairman of the Executive Committee. With Scientific Advisory Board approval, the Committee will be enlarged to five members so that on short notice a quorum of three can be convened to make emergency decisions.

10. All other matters brought before the Committee were tabled to be presented at the March Scientific Advisory Board meeting.

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

LIST OF APPLICATIONS

1003539119

Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

RENEWAL APPLICATIONS

Grant No.	Principal Investigator	Amount Requested	Years Remaining	Last Grant	Subcommittee
CANCER					
838R1	Benacerraf	$62,100	2 of 3	$62,100	Gardner, Huebner, Sommers
802R2	Kappas	$49,925		$46,130	Andervont, Bing, Huebner
758BR1 763AR2	Meier Parker	$37,950		$29,890	Andervont, Gardner, Huebner
847R1	Ryan	$55,261	2 of 3	$53,692	Huebner, Meier, Sommers
CARDIOVASCULAR					
310R12	Bing	$38,893		$29,325	Cattell, Jacobson, Sommers
844R1	Wenzel	$16,264	1 of 2	$15,849	Bing, Cattell, Sommers
519R1	Soloff	$85,016	2 of 3	$62,500	Bing, Cattell, Jacobson
PULMONARY					
808R2	Cohen	$12,487		$16,407	Gardner, Loosli, Sommers
786R2	Fudenberg	$61,737		$59,233	Loosli, Meier, Wyatt
547CR2	Guarneri	$22,944		$20,633	Jacobson, Loosli, Sommers
857R1	Kleinerman	$16,414	1 of 2	$31,600	Gardner, Loosli, Wyatt
573PR2	Loosli	$182,000		$105,989	Gardner, Jacobson, Meier
862R1	Travis	$22,237	2 of 3	$20,596	Bing, Loosli, Sommers
MISCELLANEOUS					
826R2	Dawber	$54,625		$54,338	Jacobson, Loosli, Sommers
623BR2	Essman	$21,579		$21,579	Andervont, Cattell, Meier
24R17	Larson	$49,795		$33,683	Bing, Cattell, Sommers

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

NEW AND CONTINUATION APPLICATIONS

Grant No.	Principal Investigator	Amount Requested	Anticipated Duration	Last Grant	Subcommittee
CANCER					
725CM	Chalon	$18,110	2 years	$16,192	Gardner, Loosli, Sommers
766A	Lerner	$64,385	Duration unstated	$49,495	Andervont, Gardner, Huebner
886	Sobel	$31,050	1 year		Andervont, Huebner, Loosli
896	Warren	$22,994	Duration unstated		Andervont, Gardner, Huebner
PULMONARY					
599C	Aviado	$39,984	3 years	$36,775	Bing, Cattell, Loosli
764B	Cochrane	$37,892	3 years	$31,269	Jacobson, Loosli, Wyatt
902	Galdston	$43,492	3 years		Jacobson, Loosli, Sommers
899	Greenwood	$24,035	3 years		Gardner, Loosli, Meier
741A	Lauweryns	$38,806	3 years	$5,273 (3 months)	Loosli, Meier, Sommers
903	Mendicino	$31,230	3 years		Cattell, Gardner, Loosli
897	Rosan	$54,615	3 years		Jacobson, Loosli, Sommers
901	Weinbaum	$45,332	3 years		Gardner, Loosli, Meier
EPIDEMIOLOGY					
455B	Bell	$64,000	Duration unstated	$64,000	Gardner, Jacobson, Sommers
870	Eysenck	8,625 pounds	3 years		Gardner, Huebner, Sommers
787A	Friedman	$101,100	2 years	$25,275 (3 months)	Jacobson, Loosli, Sommers

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

Grant No.	Principal Investigator	Amount Requested	Anticipated Duration	Last Grant	Subcommittee
PHARMACOLOGY					
588D	Bhagat	$36,805	3 years	$18,403 (6 months)	Bing, Cattell, Jacobson
884	Castro	$48,545	3 years		Cattell, Gardner, Jacobson
629D	Erickson	$11,891	1 year	$10,550	Bing, Cattell, Sommers
642B	Goldstein	$28,579	2 years	$7,145 (3 months)	Bing, Cattell, Meier
900	Schenker	$35,410	2 years		Bing, Cattell, Meier
894	Valzelli	$21,748	2 years		Bing, Cattell, Jacobson
776A	Vesell	$34,730	1 year	$29,555	Bing, Cattell, Loosli
MISCELLANEOUS					
880	Amplatz	$51,449	3 years		Bing, Cattell, Jacobson
898	Heimstra	$12,592	2 years		Andervont, Huebner, Meier
883	Hutchinson	$14,030	1 year		Bing, Cattell, Sommers
881	Milstein	$6,843	1 year		Andervont, Huebner, Sommers
879	Mosier	$29,513	3 years		Andervont, Gardner, Meier

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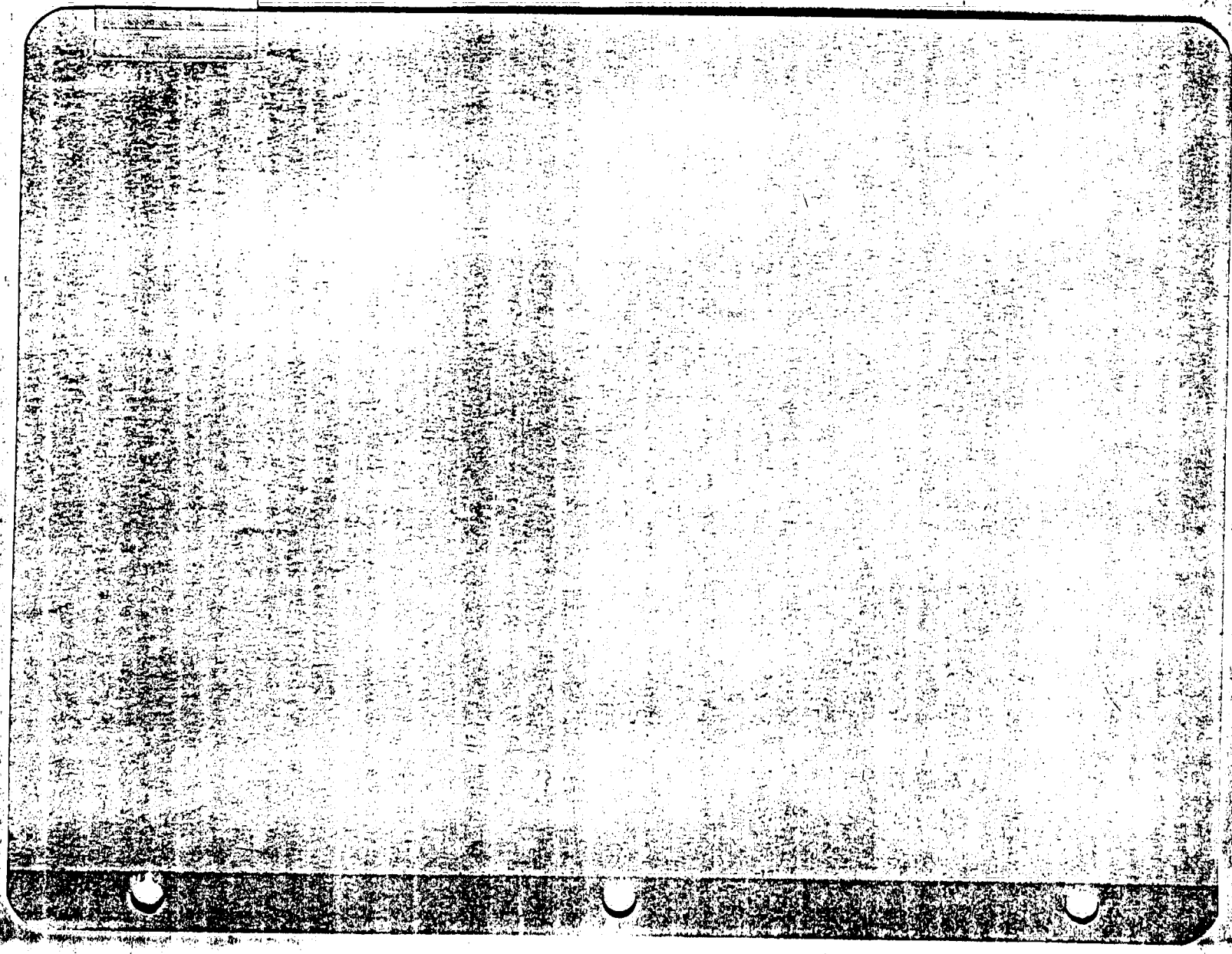
CANCER

Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

838R1 BENACERRAF

1003539124



Source: https://www.industrydocuments.ucsf.edu/docs/yyl10000

THE COUNCIL FOR TOBACCO RESEARCH – U.S.A., INC.

February 1, 1973

P=3

Grant Application No. 838R1

To: The committee comprising Drs. Gardner, Huebner and Sommers

Subject: Baruj Benacerraf, M.D., Harvard Medical School, Boston
Renewal application No. 838R1
"Control of Specific Cellular and Humoral Immune Responses to Neoplastic and Non-Neoplastic Tissues"

History

This request is for the second year of an approved three year program. The current grant is for $62,100.

This request is also for $62,100, less than originally estimated.

Documents Submitted (attached)

1. Application dated January 22, 1973.
2. Progress report 7/1/72 - 1/15/73
3. Manuscript: "Purification of Hodgkin's . . .", by Katz, Order, Graves and Benacerraf.
4. Manuscript: "Inhibition of Immune . . .", by Martz and Benacerraf.

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F.W.N.

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

Comm.
Dr. Gardner
Dr. Huebner
Dr. Sommers

CARCINOGENESIS

#838R1
#838 - 7/1/72-6/30/73

THE COUNCIL FOR TOBACCO RESEARCH—U.S.A., INC.
110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

JAN 2 2 1973

Application For Renewal of Research Grant
(Use extra pages as needed)

First Renewal ☒ Second Renewal ☐ Date: January 22, 1973

1. Principal Investigator (give title and degrees):

Baruj Benacerraf, M.D., Fabyan Professor and Chairman, Department of Pathology
David H. Katz, M.D., Assistant Professor of Pathology

2. Institution & address:

Harvard Medical School
25 Shattuck Street
Boston, Massachusetts 02115

3. Department(s) where research will be done or collaboration provided:

Department of Pathology

4. Short title of study:

Control of specific cellular and humoral immune responses to neoplastic and non-neoplastic tissues.

5. Proposed renewal date: July 1, 1973

6. How results to date have changed earlier specific research aims:

The initial results in the first six months of support by this Grant have been very gratifying and as shown in the Progress Report resulted in publications in press or to be submitted. Our results, therefore, have come up to our expectations and there is no reason to make any specific change in our stated research aims.

7. How results to date have changed earlier working hypothesis:

Our working hypotheses have not yet been fully tested but the results obtained so far greatly encourage us as to their validity.

10035391126

Source: https://www.industrydocuments.ucsf.edu/docs/vtyl0000

8. Any additional facilities now required? Describe briefly:

None

9. Any changes in personnel? Append biographical sketches of new key professional personnel:

No changes in personnel.

10. Append outline of experimental protocol for ensuing year.

11. List publications or papers in press resulting from this or closely related work. (append reprints or manuscripts not previously sent).

Katz, D.H., Order, S. W., Graves, M. and Benacerraf, B.: Purification of Hodgkin's Disease Tumor-Associated Antigens. In Press. Proceedings of the National Academy of Sciences.

Martz, E. and Benacerraf, B.: Inhibition of Immune Cell-Mediated Killing by Heparin. To be submitted to the Journal of Experimental Medicine.

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12. Summary progress report (append in standard form as separate document, unless recently submitted).

Source: https://www.industrydocuments.ucsf.edu/docs/vbvd

13. Budget for the coming year:

A. Salaries (give names or state "to be recruited")	% time	Amount
Professional (give % time of investigator(s) even if no salary requested)		
Baruj Benacerraf, M.D.	20%	-
David H. Katz, M.D.	40%	-
Technical		
M. Moran	100%	9,079.
M. Varney	50%	3,969.
M. Blanchard	100%	9,025.
W. Washington	40%	2,383.
	Sub-Total for A	24,456.
B. Consumable supplies (by major categories)		
Radioisotopes and chemicals		4,000.
Tissue culture media and materials		7,000.
Glassware and general supplies		2,000.
	Sub-Total for B	13,000.
C. Other expenses (itemize)		
Animals and animal care		16,544.
	Sub-Total for C	16,544.
	Running Total of A + B + C	54,000.
D. Permanent equipment (itemize)		
	Sub-Total for D	
E. Indirect costs (15% of A+B+C)		8,100.
	Total request	62,100.

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0227

14. Other sources of financial support:

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Studies on Experimental Cancer Immunology	National Cancer Institute National Institutes of Health - Pending	$497,506.	June 1, 1973 - May 31, 1974

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Checks payable to

-vard Medical School

Mailing address for check:

Business Office, Harvard Medical School

25 Shattuck St., Boston, Mass. 02115

Principal Investigator

Typed Name Baruj Benacerraf, M.D.

Signature Baruj Benacerraf Date 1/22/73

Telephone 617 (Area Code) 734-3300 (Number) 331 (Extension)

Responsible officer of institution

Typed Name Henry C. Meadow, Executive Secretary

Title Committee on Research and Development

Signature Henry C. Meadow Date 1/24/73

Telephone 617 (Area Code) 734-3300 (Number) 441 (Extension)

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Source: https://www.industrydocuments.ucsf.edu/docs/yty10000

10. Outline of Experimental Protocol for Ensuing Year.

1. Non-specific Enhancement of Anti-Tumor Immunity in Different Species and Experimental Tumor Systems.

The model of guinea pig L_2C leukemia employed in our original studies was chosen because of the immense challenge posed by the biological characteristics of this tumor. Thus, the rapid proliferation and disseminative nature leading to death at a relatively early and uniform time after administration makes successful immunotherapy a particularly difficult problem. This is further complicated by the fact that L_2C cells readily enter the protective environs of the central nervous system. Our significant success against such odds indicates, we believe, that such a model may be even more therapeutically efficacious in other tumor systems. We plan, therefore, to study the capacity of induced GVH reactions to enhance specific protective tumor immunity against a variety of experimental tumors, both in solid and ascitic form, in mice and rats and perhaps, hamsters. Moreover, we will adapt the model to studies of viral-induced leukemias in mice and hamsters.

In order to utilize this approach on a sound basis in species other than the guinea pig, it is essential to clearly establish appropriate conditions for induction of GVH reactions, of the type desired, in the species to be used. This is so because the magnitude of the GVH, among other less well-defined parameters, determines to a large extent the nature of the ultimate effect on the immune system of the host. Thus, although out initial observations in studies of antibody responses to hapten-carrier conjugates were concerned primarily with enhancing influences of the GVH on immune responses, further detailed analysis of the phenomenon illustrated clearly that a transient GVH reaction can suppress, with equal effectiveness, the immune system (1-3). Briefly, the most important obvious parameters in determining whether enhancement or suppression of immunity will result are those relating to 1) magnitude of the GVH, and 2) time of induction of the GVH with respect to presentation of the antigen(s) in question (1).

This point, and the importance of it, are well-exemplified by our experience over the past 1 1/2 years with the allogeneic effect in inbred mice. Studies were undertaken to determine optimal conditions for the allogeneic effect on antibody responses in mice as a prelude to utilizing this approach to enhance anti-tumor immunity in this species. However, what began as a reasonably straightforward pilot study, based on our previous knowledge of the system in guinea pigs, developed into a 1 1/2 year-long full-time research project. This resulted from the unforeseen complexities existing in inbred mice concerning major and minor histocompatibility differences, kinetic differences in optimal GVH reactivity, and perhaps also inherent genetic differences in immune system capabilities. Nevertheless, the conditions have now been developed, and carefully analyzed, for induction of appropriate GVH reactivity in a variety of mouse parental F_1-hybrid and congenic-resistant strains (1-4).

Having sufficient experience with the allogeneic effect model in mice, insofar as antibody responses are concerned, we are now able to begin to apply the appropriate conditions to studies of anti-tumor protection in this species. Toward this end, we are currently in the process of maintaining by serial transplantation several tumor lines in our own colony. These include the following tumors:

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1) Spontaneous mammary gland adenocarcinoma H2712 - C_3H host
2) Pleomorphic-cell sarcoma S37 (spontaneous mammary gland) - DBA/1 host
3) Anaplastic carcinoma dbrB (spontaneous mammary gland) - DBA/1 host
4) Methylcholanthrene-induced fibrosarcoma SaD_2 - DBA/a host

In order to standardize the system for meaningful tumor growth and survival statistics, we have been establishing dose response data utilizing freshly prepared viable single cell suspensions of tumor cells for the various lines. This will enable us to carry out experiments utilizing precise, uniformly lethal cell dose inocula for highly accurate comparisons of survival data between experimental and control groups. Over the next several months we will expand our mouse tumor lines to other available solid tumors and certain of the murine leukemias.

Our immediate plans are to begin studies of this type also in inbred rats. We are just beginning to analyze the pertinent characteristics of the allogeneic effect on antibody responses to defined antigens in rats, and to collect and serially transplant a number of transplantable rat tumor lines listed below:

1) CSE, fibrosarcoma (nickel sulfide iduced) - Fischer 344/CRBL host
2) RT-6 Cloned C_2 Glial (astrocytoma) tumor (N-nitroso-methylurea-induced) Wistar host
3) NT/W449 adencarcinoma (DES-induced) - W/Fu (female) host
4) NS 105 rhabdomyosarcoma (Nickel sulfide-induced) - Fischer 344 host
5) 13762 NM mammary adenocarcinoma (DMBA-induced) - Fischer males

The experimental plan in mice and rats will basically follow the design of the guinea pig L_2C leukemia studies. Thus, the GVH reaction, of appropriate magnitude, will be induced at varying times before, simultaneous with, or after inoculation of a lethal tumor cell inoculum and tumor growth characteristics and survival times will be compared statistically to controls. We assume that the optimal time interval of the allogeneic effect will have to be individually determined for each tumor and strain of mouse or rat involved. Once these basic parameters have been established, the systems can then be analyzed for answers to certain questions discussed in the following section.

2. Studies on the Effector Mechanisms in Host Anti-Tumor Immunity.

The studies on the protective effect of the GVH reaction on L_2C leukemia guinea pigs clearly demonstrated the participation of host immune mechanisms in the phenomenon but fell short of clearly delineating the nature of the host response. Nevertheless, certain indirect evidence derives from the guinea pig model which will be of great value when approaching the problem in inbred mice and rats.

For example, a crucial temporal relationship was found to exist between the transfer of allogeneic cells and the administration of the lethal L_2C leukemia inoculum. Thus, highly significant protection was observed in guinea pigs which received the allogeneic lymphoid cells 1, 3 or 6 days prior to lethal leukemic challenge. The protective effect was diminished somewhat in the cases where 13 days elapsed between cell transfer and leukemic challenge. In sharp contrast, transfer of allogeneic cells 21 days prior to leukemic challenge was completely ineffective in prolonging survival times, and when allogeneic cells were transferred immediately prior to challenge, only slightly prolonged survival times were observed.

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When considering which cells of the host may be primarily responsible for the anti-leukemic effect, there are essentially but two realistic possibilities: 1) the primary phagocyte cells of the RES, i.e., macrophages, and 2) the immunocompetent lymphocyte. It has been well established by others working with mice and rats that during the third week following induction of the GVH reaction in non-irradiated F_1 recipients of parental donor cells, there is a significant increase in resistance to bacterial infection (5,6). This has been attributed to heightened activation of the host macrophage population which has been shown to occur by the second or third week of the GVH response (7). It might reasonably be expected that sufficient numbers of such highly activated cells in the RES of the host could exert a non-specific anti-tumor cell effect. Indeed, Medzihradsky, working with a transplantable tumor in rats, interpreted a modest anti-tumor effect he observed in this way (8). In his system, prolonged survival was attained in F_1 recipients of parental cells transferred 14 days prior to tumor inoculation. Shorter or longer intervals between cell transfer and tumor challenge were ineffective. He suggested that the proliferation of host RES cells during the GVH reaction was responsible for this effect. In contrast, optimal anti-leukemic effects in our guinea pig system occurred much earlier in the course of the GVH response. The diminished protection observed in guinea pigs challenged with L_2C cells 13 days after allogeneic cells transfer and the failure to obtain anti-leukemic effects 21 days after transfer would argue against a primary role for hyperactive macrophages in this phenomenon. Nevertheless, a major role for these and other cells of the RES could not be excluded and lend themselves better for study in mice.

These results are, however, enlightening in the context of cellular kinetics in this phenomenon in that the transferred donor cells, themselves fully immunocompetent and thereby capable of reacting specifically against host tissues, were transplanted into a foreign environment consisting of similarly immunocompetent host lymphoid cells which not only possessed full capacity to reject the donor cells but maintained a decided advantage in numbers as well. In this circumstance we may assume, therfore, that essentially all allogeneic donor cells were rejected by the recipient in relatively short order. Assuming the 6 day interval between cell transfer and L_2C challenge to be optimal, it would seem very unlikely that sufficient numbers of donor cells, if any at all, were present in a fully functional state with the capacity to directly reject the inoculated L_2C cells. Employing the same reasoning in reverse, on the other hand, would make it a reasonable argument that in the cases of the 1-day or even, perhaps, the 3-day interval recipients, sufficient numbers of viable functional donor cells may exist to recognize and reject the foreign leukemic cells in the absence of active participation of host cells. However, if that were the case, the same effect should be true when allogeneic cells are transferred immediately prior to leukemic challenge, and this was not observed. The more appealing, and, perhaps, more likely explanation is that the critical cellular events in the expression of the protective phenomenon are ultimately performed by the host cell population which has been highly stimulated, in an as yet undefined manner, by the GVH reaction.

Evidence that the ultimate role is, in fact, performed by host cells was obtained in the guinea pig system (9). Hence, we demonstrated that protection against L_2C leukemia in this system requires the development of the GVH reaction in lymphoid organs of the host as evidenced by the failure to observe such protection in strain 2 recipients of semi-syngeneic $(2 \times 13)F_1$ hybrid donor cells. The ineffectiveness of $(2 \times 13)F_1$ lymphoid cells in affording anti-leukemia protection demonstrates that more than just antigenic stimulation of host cells is involved in the mediation of this effect. Furthermore, we found that passive

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transfer of parental strain 2 or strain 13 lymphoid cells into (2 x 13)F_1 recipients, which are genetically incapable of rejecting the parental donor cells, protects such recipients against the L_2C leukemia. This latter finding clearly demonstrated that the phenomenon depends solely on the existence of a GVH reaction and does not require the concomitant development of a host rejection response against donor lymphoid cells. The fact that parental strain 2 lymphoid cells were equivalent to strain 13 lymphoid cells in affording protection against leukemia in (2 x 13)F_1 recipients definitively excluded the possibility that donor cells directly reject the L_2C leukemia on the basis of an immune response directed at strain 2 histocompatibility antigens present of L_2C cells.

A major question, as yet unanswered definitively, concerns which subpopulation of host cells plays the primary role. As discussed above, we have considered the possible role of non-specific macrophages of the host RES. Studies on GVH reactions in mice and rats have established that during the second or third week of such reactions, a marked stimulation of RES function occurs (11). Since optimal protection in our guinea pig model occurs when leukemic challenge is made within the first week after induction of the GVH reaction, this may argue against hyperactive macrophages as the primary effector of the host. However, this argument is valid only if the stimulation of RES function during the GVH response in guinea pigs is similar in timing to that which has been demonstrated in other rodents. Since we are not aware of any definitive data on this point, such a role for macrophages in anti-leukemia protection has not been excluded. This makes an analysis of the critical time interval for anti-tumor protection by GVH in mice and rats absolutely essential.

It is more appealing, nonetheless, and perhaps more likely that immunocompetent lymphocytes of the host are the primary effector cells in this system. A suggestion of this derives from the demonstration in leukemic guinea pigs of persistent tumor immunity in 1/2 of the allogeneic cell recipients subjected to a second leukemic challenge (11). There are several possible mechanisms by which host lymphocytes may exert such a protective effect. One possibility is the production of specific anti-L_2C antibodies of a protective type which could effect destruction and/or removal of the leukemic cells. Our failure to detect specific protective antibodies by *in vivo* serum transfer and by several *in vitro* techniques argues against a role for such molecules in this phenomenon (9). However, these studies by no means rule out the production of such antibodies which may be bound to L_2C cells in host tissues, and therefore, not present in serum, or their presence in serum in quantities too small for detection by the techniques employed. More importantly, it is very conceivable that while anti-leukemia protection in guinea pigs may not involve specific antibodies, the converse may turn out to be the case in such systems in mice, rats or even humans. Alternatively, it will be of invaluable importance to determine whether an effect, perhaps suppressive, on synthesis of enhancing antibodies by the GVH can be demonstrated. This should be considerably easier to assess in tumor systems in mice and rats known to be complicated by such antibodies.

Another alternative possibility, also easier assessed in mice and rats utilizing standard *in vitro* procedures, is the production, by host lymphocytes, of non-specific cytotoxic factors as a result of the immunological attack of grafted donor cells against host tissues. Recently, such cytotoxic factors produced by host cells following induction of the GVH reaction have been demonstrated to destroy tumor cells (11). The operation of such a mechanism would not preclude the possibility that tumor-specific immuntiy may develop during the period of non-specific destruction of the tumor inoculum.

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Source: https://www.industrydocuments.ucsf.edu/docs/vfdf0000

Finally, we have favored the possibility that the anti-tumor effect in this system is a manifestation of specific cellular immunity. In a wide variety of experimental and human tumors, it appears that some degree of tumor-specific cellular immunity may exist despite the fact that the tumor continues to actively proliferate (12). The induction of a GVH reaction may serve as a potent stimulus for the initiation of marked augmentation of a specific cell-mediated immune response directed against the tumor. The observation in the L_2C leukemia system that persistent tumor immunity against leukemia existed in 1/2 of the long-term surviving allogeneic cell recipients which were subjected to rechallenge supports this hypothesis (10). Nevertheless, it was not possible to prove in that system that specific cellular immunity operates as the primary effector mechanism in this system and further investigation on this point will be absolute necessary.

A crucial point of focus in the proposed studies will be to determine whether or not specific cell-mediated anti-tumor immunity has been induced, or augmented, by the allogeneic effect in the tumor-bearing host. This can be most readily studied by adoptive cell transfer experiments in non-irradiated syngeneic recipients in both mice and rats. Studies utilizing *in vitro* techniques for evaluating specific cell-mediated immunity will also be performed toward this end. Since specific antisera are now available for selective removal of T or B lymphocytes in both of these species, it will be possible to derive a definitive answer to the question concerning the predominant effector mechanisms of anti-tumor protection in the allogeneic effect.

3. Comparative Studies on Other Non-Specific Stimulants of the Immune System.

Although the major emphasis of this proposal is to analyze in detail the efficacy of the allogeneic effect for augmentation of tumor-specific immunity, firm conclusions in this regard will necessitate full comparative evaluation of other methods known to heighten, non-specifically, the immune system in general. Once the various models proposed in the aforementioned project are established, it will be desirable and readily possible to perform such studies. Thus, the potency and comparative effectiveness of stimuli such as the T cell mitogens, concanavalin A and *E. coli* lipopolysaccharide (endotoxin), infectious agents such as Listeria monocytogenes and derivatives thereof (i.e., cell extracts), other non-specific adjuvants such as polyadenylic:polyuridylic acid (Poly A:U), and beryllium salt, and macrophage-activating substances can be thoroughly evaluated. Effects of the above on stimulation of tumor immunity will be assessed for cell-mediated as well as humoral (enhancing vs cytotoxic antibodies) components both *in vivo* and *in vitro*, and, of course, the respective roles of lymphocytes and macrophages will be analyzed.

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Source: https://www.industrydocuments.ucsf.edu/docs/ytwf0053

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Source: https://www.industrydocuments.ucsf.edu/docs/vyln0000

Mechanism of Specific Cytolysis of Malignant Target Cells by Immune Thymus-Derived Lymphocytes.

There are a number of *in vitro* models for the several mechanisms involved in graft or tumor rejection *in vivo* (1,2). These involve cytotoxicity to target cells mediated by effector cells from lymphoid tissues, as first described by Govaerts (3). Such cell-mediated toxicity can be divided into two categories, according to the type of effector cells responsible: the first category includes cytotoxicity effected by non-thymus-derived cells, such as "B" lymphocytes (4,5) or monocyte-macrophage cells (6,7,8). The second concerns cell-mediated killing effected directly by thymus-derived lymphocytes. The first type of killing most likely depends upon antibodies (1,5) which may be released locally by antibody-producing "B" lymphocytes (plasma cells) in some *in vitro* systems. Since tumor and graft rejection is transferred by cells but not by serum (9) we feel that cytotoxicity of the second type, or related activities of the thymus-derived effector cells, must be crucial in specific tumor or graft rejection. Therefore, we shall be concerned primarily with this second phenomenon.

Using the mouse DBA/2 P815 mastocytoma as the target for allo-immunized killers, it has been shown that thymus cells can give rise to killer cells (10) and that cells bearing the theta antigen are necessary for this cytolytic activity (11). Thymus-derived lymphocytes can probably kill without "help" from bone marrow-derived cells, since attempts to remove the latter have not diminished killing activity (12,13,14). Specific killer cells can be produced *in vitro* by culturing lymphoid cells with antigen-bearing cells (15-18) and killers produced in this way are the products of cell division (15,19). The killer cell bears antigen recognition sites which allow it to be specifically removed from a lymphoid population by adhesion to antigen-bearing fibroblast monolayers, and subsequently recovered from the monolayer (20,21). Covering up the antigenic sites on the target cell with alloantisera inhibits lysis (22,23). Killing by these effector cells is relatively rapid: a majority of the target population can be lysed in less than 1 hour (12,13). In this system, non-specific ("bystander") target cells, when mixed among immune lymphocytes and specific targets, are relatively unharmed by the closely adjacent killing process (13,15, 24-26); the killers themselves are not lysed (27,28) and are able to kill a second population of targets at the same or greater rate than the first (12,28). Target cell killing progresses in medium containing heat-inactivated serum, and addition of exogeneous complement does not affect the rate of cytolysis (24,29,30). The killing process, however, requires some heat-stable non-dialyzable serum components (13) as well as calcium or magnesium ions (31).

In spite of the advances just described, much remains to be elucidated about specific target cell lysis by immune thymus-derived lymphocytes. The questions can be grouped in three categories:

I. What is the developmental origin, the pathway of differentiation, and the fate of the thymus-derived killer lymphocyte?

   a) What is the immediate precursor of the effector cell? How much proliferation is required? And where does this proliferation and differentiation take place *in vivo*?

   b) What is the lifetime of the effector cell?

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c) Is the effector cell in a terminal state of differentiation?

d) Are there killer memory cells? Is there a secondary killer response?

e) Are there more than one type or state of thymus-derived killer lymphocytes (26,32)?

f) What is the relationship between the killer cell and cells effecting other thymus-derived functions (such as release of MIF, graft vs host reactions, mixed lymphocyte reactions)?

II. What is the mechanism by which the sensitized thymus-derived lymphocyte kills the target cell? Even less is known about this mechanism than about the nature of the killer cell.

a) How long does the killing of a single target cell require? Is this time constant or variable? If variable, does it depend on the state of the killer or the target? Is the difference in killing rate for different targets bearing the same alloantigens (12) a function of the speed of each killing event, or the rate at which the killer can move from one target cell to the next?

b) Does the killing of several targets by one killer (12,28) occur in sequence or in parallel?

c) Do cell membrane movements or killer cell locomotion play any role in killing?

d) What are the steps in killing, what is their sequence, and how long does each take?

e) What proteins or other substances must be synthesized (23) after the killer is confronted with specific antigen, and what is their role?

f) Do the soluble cytotoxins found in culture supernatants (33,34) play a role in cell-mediated killing?

g) What serum factors are required and what is their role? Are they equivalent to those required for survival, movement or DNA synthesis of fibroblasts (35)?

h) Are divalent cations, serum factors, and 37°C temperature required throughout the killing process or only at certain critical steps, perhaps each with different requirements?

i) Is osmotic disequilibrium across the cell membrane necessary for completion of lysis, as it is for complement mediated lysis (36)?

j) What accounts for the great strength of the adhesion between killer and target?

k) What is the ultrastructural morphology of the killer, and its interaction with the target at various stages during killing?

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III. What is the role of sensitized thymus-derived killer lymphocytes in tumor or graft rejection in vivo? This is the most difficult question to resolve, since it depends on a better understanding of I and II above. We must learn how many of each kind of effector cell are present in the tumor tissue during the stages of rejection (which will require enumerative assays for each functional class of cell), how and why they got there, and their relative contributions to death of the tumor tissue. Ultimately, it should be possible to manipulate the effector cell system so as to potentiate damage to tumor tissue.

Experimental Design

Initially, we plan to focus our research efforts largely on the mechanism of specific target cell killing by sensitized thymus-derived lymphocytes (category II above). The killing is undoubtedly a multistep process (30) which depends initially upon the interaction of antigen on the target cell membrane with specific lymphocyte receptors.

Experimentation is in progress in our laboratory to attempt to resolve this sequence of steps through studies utilizing inhibitors. We have found (37) that heparin (previously known to interfere with several forms of cellular immunity [38-40])and other highly charged polyanions retard a rapid, early killer-target interaction necessary for killing. Once this interaction has occurred, it cannot be reversed by subsequently added heparin, and lysis proceeds to completion at the normal rate (see progress report).

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Source: https://www.industrydocuments.ucsf.edu/docs/xyd0000

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Source: https://www.industrydocuments.ucsf.edu/docs/vtyl0000

Section 12 - Summary Progress Report

CIR Grant 838

Progress Report No. 1

Dates Covered:
7/1/72 - 1/15/73

Investigator

Baruj Benacerraf, M.D., Fabyan Professor of Comparative Pathology

Institution

Harvard Medical School
25 Shattuck Street
Boston, Massachusetts 02115

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1. Experiments Designed to Enhance Protective Anti-Tumor Immune Responses.

a) BCG in mice

Studying a neuroblastoma which arose spontaneously in the A mouse strain, experiments were carried out to investigate the effect of live BCG on the rejection of this tumor in the syngeneic host. This neuroblastoma is uniformly lethal for adult A strain mice in a dose of 10^2 tumor cells in about 15 days. Injection of live BCG with the tumor inoculum protected the mice against a tumor inoculum 100 times larger (10^4 cells). To explore the mechanism of this effect and more precisely whether BCG administered with the tumor was able to increase specific tumor immunity, the mice which survived the 10^4 tumor cell challenge and BCG were reinoculated with 5×10^3 neuroblastoma cells at the same time as control A strain mice. A very significant increase in survival time following this inoculum was observed in the BCG treated group compared to the control group. These findings indicate some increase in tumor immunity as a consequence of BCG administration with tumor cells in mice.

b) The allogeneic effect caused by graft versus host reactions.

After the experimental conditions required for the injection of allogeneic immunocompetent cells to exercise adjuvant effects had been ascertained in mice and rats, experiments have been initiated to test the protective action of the allogeneic effect on the rejection of solid syngeneic tumors in these two species. The following tumors have been studied in mice:

Mammary carcinomas in C_3H and DBA_2 strains

Rhabdomyosarcomas in DBA_2 strain

Fibrosarcoma in DBH_1 strain

In rats two tumors are being investigated, a mammary adenocarcinoma induced by nickel iodide in the Fisher 344 strain and a glioblastoma, which arose spontaneously in the Wistar-Furth strain. GVH reactions are induced by the injection of appropriate numbers of allogeneic immunocompetent cells. So far in preliminary experiments,

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Source: https://www.industrydocuments.ucsf.edu/docs/yfyl0000

GVH reactions, or the allogeneic effects, were successful in decreasing very significantly the growth of transplanted glioblastoma in its syngeneic host. Further experiments with all the above mentioned tumors are in progress.

2. Purification of Hodgkin's Disease Tumor Associated Antigens. (Proc. Nat. Acad. of Sciences (In press), preprint attached.

These studies designed to characterize the antigens associated with Hodgkin's Disease were carried out in collaboration with Dr. Order of the Department of Radiation Therapy.

Two antigens which exist in high frequency in tumor tissues of patients with Hodgkin's Disease have been obtained in relatively concentrated form. Extracts of Hodgkin's spleen tumor tissue when subjected to chromatography on Sephadex G-200 separate into three major protein peaks of which only the first (peak I) possesses the predominant antigenic activities associated with the disease. Antigenic analysis performed with hyperimmune rabbit antisera obtained after repeated immunizations with peak I proteins demonstrated that this fraction contained both F and S antigens associated with Hodgkin's Disease and small contaminant amounts of an antigen associated with normal lymphocytes. The tissue distribution patterns of the Hodgkin's Disease tumor-associated antigens suggest that they both originate in lymphoid tissues and that F antigen may represent a product of reactive lymphocytes while S antigen may be a dedifferentiation antigen expressed in very immature lymphocytes.

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Source: https://www.industrydocuments.ucsf.edu/docs/vyvl0000

3. Studies on the Mechanism of Immune Cytolysis by Sensitized Thymus Derived Cells.
Inhibition of immune cell-mediated killing by Heparin.
(to be submitted to the J Exp Med) preprint attached.

Heparin retarded the cell-mediated lysis of mouse ascitic tumor cells or normal spleen cells by effector spleen cells from allo-immunized mice. The retardation was immediately reversible by removal of the heparin. Heparin reduced the rate of cell lysis without preventing the eventual complete destruction of the target cell population. The completion of killing was not due to loss of heparin activity or desensitization of the killer cells to heparin. At a given concentration of heparin, the amount of inhibition depended on the target cell type employed, but was relatively independent of the speed with which lysis progressed. Retardation was independent of divalent cation concentration and serum concentration, and was not affected by depletion of red blood cells or adherent cells. Heparin did not inhibit incorporation of tritiated thymidine in mixed lymphocyte cultures, and hence was not non-specifically toxic for lymphocytes. The dose responses of killing to three other highly sulfated polymers were similar to that for heparin. This indicates that the inhibition is due to the high charge density and polyanionic character of heparin, and not to an impurity. The anti-coagulant activity of these polyanions was not correlated with their inhibitory activity for cell-mediated killing.

When heparin is added to cultures after about 10% specific release of chromium has occurred, it has virtually no effect on subsequent killing. This is not due to desensitization of the killer cells to heparin as a result of the previous killing experience. Hence, there is an early heparin-sensitive killer-target interaction which, once it has occurred, cannot be reversed by heparin. This sensitive interaction seems likely to be the formation of a close contact between the killer and target cells.

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Source: https://www.industrydocuments.ucsf.edu/docs/jfpb0000

802R2 KAPPAS

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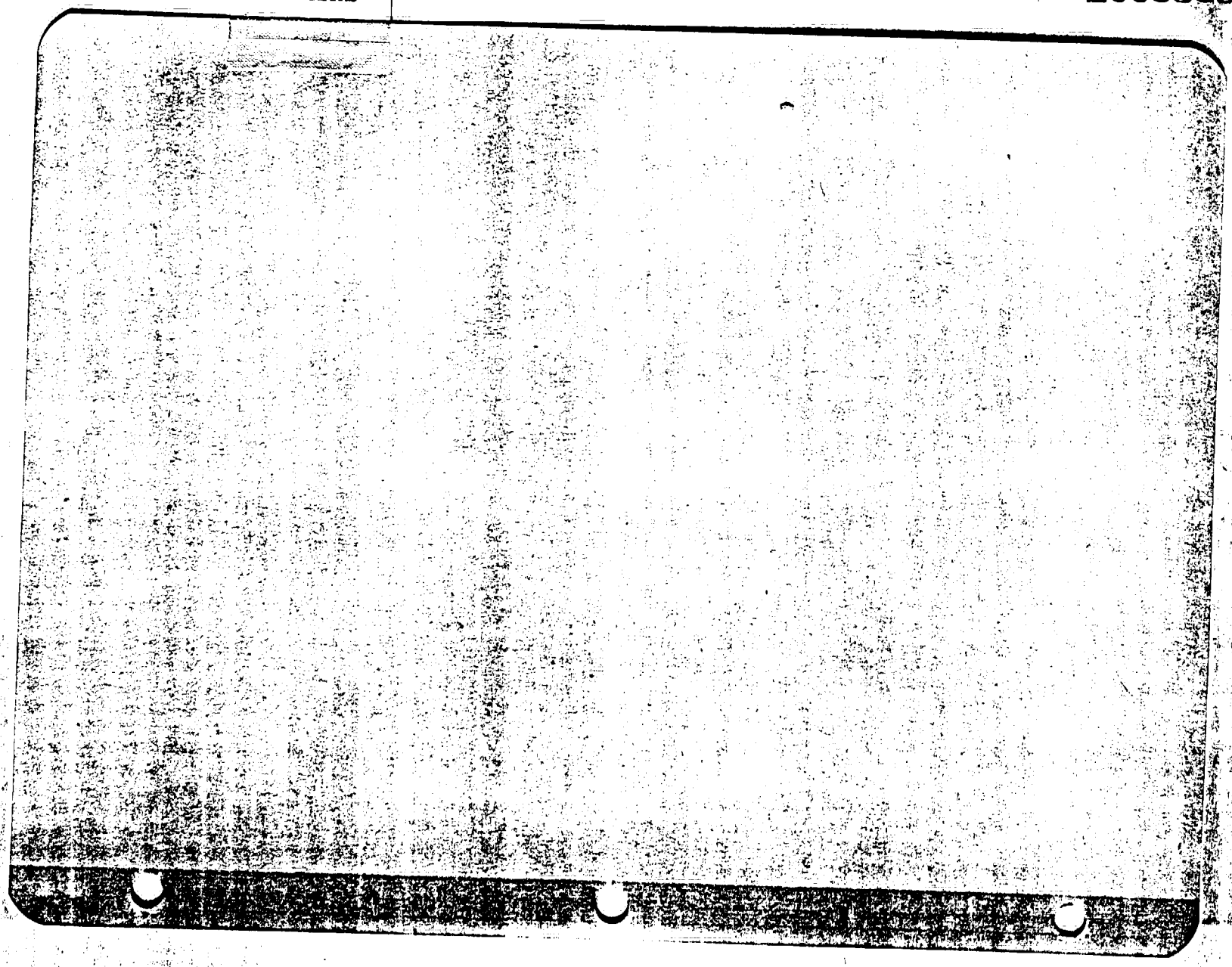

Source: https://www.industrydocuments.ucsf.edu/docs/Myl0000

THE COUNCIL FOR TOBACCO RESEARCH – U.S.A., INC.

February 1, 1973 P-1

Grant Application No. 802R2

To: The committee comprising Drs. Andervont, Bing and Huebner

Subject: Attallah Kappas, M.D., The Rockefeller University, New York
Second renewal application No. 802R2
"Tobacco Smoke: Effects on Detoxification Mechanisms"

History

This request is for the third year of a three year project approved by the SAB. The grant now in effect is for $46,130.

The pending request is for $49,925, against an earlier estimate for this year of $46,000.

Documents Submitted (attached)

1. Application dated January 30, 1973.

2. Appendix: Progress Report and Investigations Planned for 1973-74.

Comment

Dr. Kreisher has visited this grantee to help with the smoke exposure device.

At our request, Dr. Kappas has promised to send copies of the manuscripts listed under Item 11 on page 2 of the application.

F.W.N.

FWN:wg
Encls.

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Source: https://www.industrydocumentslibrary.ucsf.edu/tobacco/

Comm.
Dr. Andervont
Dr. Bing
Dr. Huebner

CARCINOGENESIS

#802R2
#802R1 - 7/1/72-6/30/73
#802 -7/1/71 -6/30/72

THE COUNCIL FOR TOBACCO RESEARCH–U.S.A., INC.
110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

RECEIVED
JAN 31 1973

Application For Renewal of Research Grant
(Use extra pages as needed)

First Renewal ☐ Second Renewal ☒

Date: January 30, 1973

1. Principal Investigator (give title and degrees):

Attallah Kappas, M.D. - Professor & Senior Physician

Alvito P. Alvares, Ph.D. - Assistant Professor

2. Institution & address:

The Rockefeller University
66th Street & York Avenue
New York, N. Y. 10021

3. Department(s) where research will be done or collaboration provided:

Laboratory of Metabolism and Pharmacology
Rockefeller University Hospital

4. Short title of study:

Tobacco Smoke: Effects on detoxification mechanisms

5. Proposed renewal date: July 1, 1973 - June 30, 1974

6. How results to date have changed earlier specific research aims:

There has been no basic change with respect to the research aims outlined in the prior progress report. The only additional work planned relates to the study of the effects of coal tar therapy in psoriatic individuals. In addition, the delayed delivery of the Walton Smoke Machine has necessarily led to a deferral of certain animal studies; these are now in progress and we plan to complete them in the coming year.

7. How results to date have changed earlier working hypothesis:

There has been no change in our original working hypothesis.

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Source: https://www.industrydocuments.ucsf.edu/docs/ylyl0000

8. Any additional facilities now required? Describe briefly:

None required.

9. Any changes in personnel? Append biographical sketches of new key professional personnel:

None made. The budget for FY 1973-74 reflects anticipated salary increases for Dr. Alvares and his technical assistant.

10. Append outline of experimental protocol for ensuing year. See appendix.

11. List publications or papers in press resulting from this or closely related work. (append reprints or manuscripts not previously sent).

1. W. Levin, A. H. Conney, A. P. Alvares, I. Merkatz and A. Kappas: Induction of benzo[α]pyrene hydroxylase in human skin. Science 176: 419-420, 1972.

2. A. P. Alvares, A. Kappas, W. Levin and A. H. Conney: Inducibility of benzo[α]pyrene hydroxylase in human skin by polycyclic hydrocarbons. Clinical Pharmacology and Therapeutics, In press, 1973.

3. A. P. Alvares, S. Leigh, A. Kappas, W. Levin and A. H. Conney: Induction of aryl hydrocarbon hydroxylase in human skin. Proceedings of the Second International Symposium on Microsomes and Drug Oxidations to be published in Drug Metabolism and Disposition, In press, 1973.

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12. Summary progress report (append in standard form as separate document, unless recently submitted). See appendix.

Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

13. Budget for the coming year:

A. Salaries (give names or state "to be recruited")

Professional (give % time of investigator(s) even if no salary requested)	% time	Amount
Dr. A. Kappas	25%	-----
Dr. A. P. Alvares	100%	$20,000.
Technical		
1 Technical Assistant	100%	10,000.
	Sub-Total for A	30,000.

B. Consumable supplies (by major categories)

Chemicals and isotopes	3,500.
Laboratory animals	2,500.
	Sub-Total for B 6,000.

C. Other expenses (itemize)

Travel	500.
Preparation of figures, xerox, reprints, publication costs.	1,500.
Upkeep of animal facility	1,500.
	Sub-Total for C 3,500.
	Running Total of A + B + C $39,500.

D. Permanent equipment (itemize)

Aminco-Chance Split Beam dual wavelength Spectrophotometer (Final installment attributable to CTR grant #802).

Sub-Total for D	4,500.
E	5,925.
Total request	$49,925.

E. Indirect costs (15% of A+B+C)

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Source: https://www.industrydocuments.ucsf.edu/docs/yjfl0000

14. Other sources of financial support:

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
1. Entire salary of Dr. A. Kappas, secretary and office expenses provided for by Rockefeller University.			
2. Effects of lead poisoning on hepatic heme synthesis (ES-00621) - Dr. A. Kappas, Principal Investigator.	ES-00621	$59,684.	1973-74
3. General research support.	Hoffmann-LaRoche, Inc.	$15,000.	1973-74

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
1. We plan to submit a renewal application for NIH grant ES-00621 in the autumn of 1973.	NIH		

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name Dr. Attallah Kappas

Signature [signature] Date 1/30/73

Telephone 212 (Area Code) 360-1286 (Number) (Extension)

Checks payable to

e Rockefeller University

Mailing address for check:

Miss O. Margery Pedersen, Chief Accountant
The Rockefeller University, New York, N. Y. 10021.

Responsible officer of institution

Typed Name Dr. Frederick Seitz

Title President

Signature [signature] Date 1/30/73

Telephone 212 (Area Code) 360 1234 (Number) (Extension)

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Source: https://www.industrydocuments.ucsf.edu/docs/yty10000

APPENDIX: PROGRESS REPORT AND INVESTIGATIONS PLANNED FOR 1973-74

Tobacco smoke: Effects on detoxification mechanisms

1. Induction of aryl hydrocarbon hydroxylase in human skin:

The aryl hydrocarbon hydroxylase enzyme system which hydroxylates polycyclic hydrocarbons has previously been shown to be present in human liver and placenta. In studies completed to date, we have utilized the tissue culture system and grown human skin in presence and in absence of the polycyclic hydrocarbon, benz[a]anthracene. We have established for the first time the presence of aryl hydroxylase in human skin. We have also studied the properties of this enzyme and results show that its properties in skin and liver are similar. The skin hydroxylase, like the hepatic hydroxylase, has an absolute requirement for NADPH and molecular oxygen and the hydroxylation reaction has a pH optimum of 7.4. The microsomal hemoprotein, cytochrome P-450 was also shown to be a key component of the skin hydroxylase system. When skins were incubated in a growth medium containing benz[a]anthracene, 2- to 5-fold increases in the amount of benzo[a]pyrene hydroxylase were observed. In skins obtained from 13 individuals, significant variations in individual inducing capacities were observed. We have postulated that these differences in the degree of induction might be due to genetic differences and that individuals with a lower capacity to metabolize carcinogens may be more susceptible to the carcinogenic action of polycyclic hydrocarbons ingested orally, through inhaled smoke or through skin absorption. Previous studies have demonstrated individual differences in the ability of humans to metabolize several drugs.

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A comparison of the hydroxylase activities in human, rat and mouse skins was also carried out. The basal levels of the enzyme in man are lower than in the mouse or rat and the inducibility of the enzyme is greater in human skin than in the mouse or rat skins. A time-course study showed that the maximum induction of benzo[a]pyrene hydroxylase in skin, incubated in the presence of benz[a]anthracene, occurred between 16 to 24 hours.

Human skin is one of the largest organs of the body. The skin of an average man has been estimated to weigh about 3 times as much as his liver, the principal detoxifying organ in the body. The presence of hydroxylase activity and the sheer size of the skin pool suggests a significant role of skin in detoxification processes in man. Since skin biopsy is relatively easy to perform, biopsy samples may be of predictive value in determining the carcinogenic hazard in people exposed to polycyclic hydrocarbons. The results of the above studies have been the subject of 3 publications, which are cited at the end of this report.

2. Studies on patients treated with coal tar:

Psoriasis is a chronic skin disease characterized by scaling erythematous plaques which may involve single body areas on the entire integuement. The disease is not curable but one of the most effective and widely used therapeutic modalities is based on a regimen described by Goeckerman in 1925. Crude coal tar in a 5 to 10% concentration in petrolatum is applied to all the skin lesions and left in place for 24 hours. The material is then removed with mineral oil and the entire body exposed to ultraviolet radiation sufficient to produce a moderate erythema. This procedure is repeated daily until the lesions have cleared, generally in 2 to 3 weeks. Since crude

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coal tar is a mixture of a number of aromatic hydrocarbons, including benzo[a]pyrene, we have initiated studies designed to assess the effect of skin application of crude coal tar on drug metabolism in man and on benzo[a]pyrene hydroxylase activity in skin biopsy samples. To study drug metabolism, antipyrine and phenacetin, two widely used antiinflammatory and antipyretic agents have been selected for study. Phenacetin and antipyrine were chosen for these studies since smoking has been shown to lower plasma concentrations of phenacetin and since inducers of drug metabolism cause a decrease in antipyrine half-lives. The plasma half-lives of these two drugs are determined prior to and following treatment of psoriatic patients with coal tar, thus each subject serving as his own control. These studies are being presently carried out in collaboration with Dr. David Bickers, a resident dermatologist at the Skin and Cancer Unit, Department of Dermatology, New York University School of Medicine.

3. Effects of cigarette smoking on the disposition of drugs in man:

Studies have been carried out to determine the effects of smoking on the metabolism of drugs in man. Phenylbutazone and antipyrine have been used as model drugs metabolized primarily by the hepatic microsomal mixed function oxidase system. Following administration of these drugs, the disappearance rates of these drugs from plasma (half-life) provides a measure of the functional capacity of the microsomal enzyme system that metabolizes drugs, other foreign compounds and normal body constituents. In studies completed to date in 20 normal healthy volunteers, smokers showed no significant difference in the metabolism of these model drugs when compared with the half-lives obtained in non-smokers.

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During the course of these studies in adults, we also investigated the half-lives of these drugs in children. Contrary to data obtained with animals where the rates of drug metabolism are much slower in young animals in comparison with adult animals, young children showed a significantly faster rate of drug disposition when compared to adults when the same dosage of antipyrine or phenylbutazone was administered on a per kilogram basis. Thus, for example, the mean half-life of antipyrine in 10 children was 6.6 hours compared with a mean half-life of 13.6 hours obtained in 10 adults. This finding is of major therapeutic significance and is the subject of a manuscript being prepared for publication.

4. Studies on cytochrome P-450 and P-448:

Inducers of hepatic drug-metabolizing enzymes have been categorized into two general groups of chemicals. One group of inducers is mainly drugs, for example phenobarbital, dilantin, etc. A second group of inducers comprise the polycyclic hydrocarbons, such as benzo[a]pyrene and 3-methylcholanthrene. A major difference between these two classes of inducers is their effect on cytochrome P-450. Phenobarbital is a potent inducer of cytochrome P-450; in contrast, polycyclic hydrocarbons induce the formation of a spectrally and catalytically different hemoprotein, cytochrome P-448. Studies on these two cytochromes have been carried out in the laboratory in attempts to isolate and purify these microsomal hemoproteins. To date we have achieved a 6-fold purification of these cytochromes. We have also in the course of our studies, discovered the presence of a new form of cytochrome P-448 which though

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Source: https://www.industrydocuments.ucsf.edu/tobacco/docs/

spectrally similar to the P-448 induced by polycyclic hydrocarbons differs in catalytic properties from the hemoprotein induced by these hydrocarbons. This new P-448 is induced by a hitherto unknown class of inducers, the polychlorinated biphenyls (PCB's). The PCB's which are newly recognized as environmental pollutants have recently been discovered to be among the most ubiquitous chemical contaminants of animal and human tissues. In excess of 50% of humans tested have residues of these compounds in their adipose tissues. The sources of this contamination are not clear. The striking differences in the enzymatic properties of cytochrome P-448 induced by polychlorinated biphenyls, of cytochrome P-448 induced by polycyclic hydrocarbons, and of cytochrome P-450 induced by drugs is the subject of a manuscript recently submitted for publication in the Proceedings of the National Academy of Sciences. This study is the first demonstration of a class of chemical substances which are structurally different from the polycyclic hydrocarbons and which induce cytochrome P-448.

5. Enzyme induction following exposure to cigarette smoke using the Walton Smoke Machine:

In August, 1972, we received the Smoke Exposure Machine from the Council of Tobacco Research, U.S.A. Studies are now in progress on the effects of cigarette smoke from the University of Kentucky Reference Cigarettes on the induction of aryl hydrocarbon hydroxylase in rat lung, liver, skin and intestines. Rats are conditioned for a week in the machine prior to exposure to cigarette smoke. Varying times of exposure, acute as well as long-term chronic studies are being carried out. Following exposure, livers are removed and drug-metabolizing enzyme activities,

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cytochrome P-450 levels and NADPH-cytochrome c reductase activities are determined. In other tissues, only benzpyrene hydroxylase activities and cytochrome P-450 levels are determined because of the sensitivities of these assay systems. We plan to carry out similar studies, in the coming year, using pregnant rats and studying these effects on the maternal host as well as on the fetuses.

Presentations (February 1, 1972 - January 31, 1973)

1. Induction of aryl hydrocarbon hydroxylase in human skin by A. P. Alvares at the Second International Symposium on Microsomes and Drug Oxidations, Stanford University, Stanford, Calif.; July 31, 1972.
2. The induction of a carcinogen-metabolizing enzyme in human skin by A. Kappas at the Annual meeting of the American Clinical and Climatological Association, Ponte Vedra Beach, Florida; October 31, 1972.
3. Alteration in hepatic microsomal hemoproteins induced by polycyclic hydrocarbons and polychlorinated biphenyls by A. P. Alvares at the Department of Pharmacology, School of Medicine, New York University, New York, N. Y.; January 12, 1973.

Publications (February 1, 1972 - January 31, 1973)

1. W. Levin, A. H. Conney, A. P. Alvares, I. Merkatz and A. Kappas: Induction of benzo[α]pyrene hydroxylase in human skin. Science 176: 419-420, 1972.

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Source: https://www.industrydocuments.ucsf.edu/docs/tyfd0000

2. A. P. Alvares, A. Kappas, W. Levin and A. H. Conney: Inducibility of benzo[α]pyrene hydroxylase in human skin by polycyclic hydrocarbons. Clinical Pharmacology and Therapeutics, In press, 1973.

3. A. P. Alvares, S. Leigh, A. Kappas, W. Levin and A. H. Conney: Induction of aryl hydrocarbon hydroxylase in human skin. Proceedings of the Second International Symposium on Microsomes and Drug Oxidations to be published in Drug Metabolism and Disposition, In press, 1973.

4. A. P. Alvares, D. R. Bickers and A. Kappas: Polychlorinated biphenyls: A new type of inducer of cytochrome P-448 in the liver. Proceedings of the National Academy of Sciences, submitted for publication, 1973.

Manuscript in Preparation

1. A. P. Alvares S. Kapelner, D. R. Bickers and A. Kappas: Age differences in the rates of drug metabolism in man: Studies with antipyrine and phenylbutazone.

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THE COUNCIL FOR TOBACCO RESEARCH–U.S.A., INC.
110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

P = 5

Application For Renewal of Research Grant

(Use extra pages as needed)

First Renewal ☒ Second Renewal ☐ Date: 12 February 1973

1. Principal Investigator (give title and degrees): Hans Meier, D.V.M., Dr. med. vet., M.R.S.H., Senior Staff Scientist, Richard R. Fox, Ph.D., Staff Scientist

2. Institution & address: The Jackson Laboratory, Bar Harbor, Maine 04609

3. Department(s) where research will be done or collaboration provided: Hamilton Laboratory, West and Virus-Leukemia Laboratory

4. Short title of study: Oncogenesis in the rabbit: genetic susceptibility, vertical transmission of virus and environmental influences

5. Proposed renewal date: 1 July 1973

6. How results to date have changed earlier specific research aims: The results obtained to date have not changed our original basic aims. However, several new approaches are proposed.

7. How results to date have changed earlier working hypothesis: Our earlier working hypothesis remains unchanged.

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

8. Any additional facilities now required? Describe briefly: New stainless steel cages are required for replacement of old galvanized cages.

9. Any changes in personnel? Append biographical sketches of new key professional personnel: No personnel changes are contemplated.

10. Append outline of experimental protocol for ensuing year. See attached protocol.

11. List publications or papers in press resulting from this or closely related work. (append reprints or manuscripts not previously sent). See attached.

12. Summary progress report (append in standard form as separate document, unless recently submitted). Included in objectives or specific aims.

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Source: https://www.industrydocuments.ucsf.edu/docs/AnyID#

13. Budget for the coming year:

A. Salaries (give names or state "to be recruited")	% time	Amount
Professional (give % time of investigator(s) even if no salary requested)		
Hans Meier, Project Director	5	-
R.R. Fox, Project Co-Director	20	$ 3,945
Technical		
D.D. Crary, Professional Assistant	10	1,065
R.F. Norberg, Professional Assistant	15	1,250
Eugene Farrin, Research Assistant	20	1,500
Animal Caretaking		4,000
Secretarial Assistance		500
	Sub-Total for A	$12,260
Employee benefits: 17.5%		2,145
B. Consumable supplies (by major categories)		
Chemicals, glassware, instruments		1,600
Food and bedding		1,700
	Sub-Total for B	$ 3,300
C. Other expenses (itemize)		
Domestic travel to scientific meetings (2 investigators)		500
Publication costs		300
Art, Photo, and Replicating Service		200
	Sub-Total for C	$ 1,000
	Running Total of A + B + C	$16,560
D. Permanent equipment (itemize)		
Thirty (30) Bussey Model 1491S cages		1,800
	Sub-Total for D	$ 1,800
E. Indirect costs (15% of A+B+C)	E	$ 2,805
	Total request	$23,310

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Source: https://www.industrydocuments.ucsf.edu/docs/yzb00d

14. Other sources of financial support:

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
ncogenesis in the rabbit: genetic usceptibility, vertical trans-ission of virus, and environ-ental influences ee attached.	The Council for Tobacco Research, U.S.A.	$21,330*	6/1/72-5/31/73

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Natural occurrence of RNA tumor viru (genomes) and host-gene con l of their expressions See attached.	National Cancer Institute	$412,529	5/1/73-4/30/74

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name Hans Meier

Signature Hans Meier Date 5/12/73

Telephone 207-288-3373 273
Area Code Number Extension

Checks payable to The Jackson Laboratory
tin C. Carter, Financial Manager

Mailing address for check:
Bar Harbor, Maine 04609

Responsible officer of institution

Typed Name Austin C. Carter

Title Financial Manager

Signature [signature] Date 12 Feb 73

Telephone 207-288-3373 227
Area Code Number Extension

10035391

Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

14. Other sources of financial support:

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
tural occurrence of RNA tumor vir- (genomes) and host-gene control their expressions	National Cancer Institute	$395,064	5/1/72-4/30/73
reditary diseases and variations the rabbit	NIH General Research Support grant to the Jackson Laboratory	63,836*	7/1/72-6/30/73
bbit inbred and mutant stocks source	NIH Division of Research Resources	25,000*	1/1/73-12/31/73

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
reditary diseases	National Institute of General Medical Sciences	$ 50,487*	4/1/73-3/31/74
bbit inbred and mutant stocks source	NIH Division of Research Resources	90,015*	1/1/74-12/31/74
nes, viruses, and cancer	National Cancer Institute	69,327*	4/1/73-3/31/74

*Direct costs

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Checks payable to

Mailing address for checks

Principal investigator

Typed Name

Signature Date

Telephone
Area Code Number Extension

Responsible officer of institution

Typed Name

Title

Signature Date

Telephone
Area Code Number Extension

10035391/2

Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

Details of Experimental Design and Procedures (from page 2):

Introduction and background. In our applications (1970-72) for CTR Research Grant 758, we stated the aims of our studies in detail. Clearly, the work that we proposed in our 1972 application could not be completed within the period of the 1 year for which we received funding; at least 2 additional years of work and support are required. Thus, this application is for continuance of studies currently underway.

The overall goals of our studies remain basically as proposed previously:

(1) We have observed 75 cases of lymphosarcoma in a small breeding colony of strain WH rabbits within a few years, and affected animals of both sexes were found in each of several generations. Because of the unusual aggregation of cases of lymphosarcoma among WH rabbits, we wish to investigate the host genetic factors conferring susceptibility to lymphosarcoma, the mode of inheritance or transmission, the possibility of a vertically transmitted virus, and the environmental influence that may modify incidence and pathogenesis of lymphosarcoma.

(2) Another strain of rabbits, strain X, which is genetically related to the WH strain, is characterized by a high incidence of immune hemolytic anemia (60 cases); thymoma occurs as well. We wish to determine the pathogenesis of both immune hemolytic anemia and thymoma, and investigate their possible etiological relationship.

We want to find out the mode of inheritance or transmission of immune hemolytic anemia and thymoma in strain X rabbits so that we may evaluate the possibility of a common hereditary basis for all conditions in both strain X and WH, since the two strains are genetically related. The various clinical or phenotypic expressions probably derive from differences in the genetic background of the two strains.

(3) We believe that all three conditions are caused by a vertically transmitted virus analogous to the C-type RNA viruses occurring in a number of vertebrates, including man. The outcome of viral-host interaction depends to major degrees on host genetic factors, but it may be modified by environmental influences.

Scientific progress during tenure of current grant. A brief background and resume of work accomplished this far is pertinent to the studies proposed in this application. Detailed reports of our accomplishments have been submitted (Progress Reports 1, 2, and 3).

(1) Hereditary lymphosarcoma in strain WH rabbits.

(a) Genetic and pathologic studies. In 1962, we first observed a lymphosarcoma in the WH strain of rabbits at the Jackson Laboratory. Since 1965, we have observed 74 more lymphosarcomas in this strain. Because of this unusual aggregation of lymphosarcomas, we have analyzed the pedigrees of the affected animals. Lymphosarcoma in the WH strain of rabbits was found to have a hereditary basis. The inheritance indicated an autosomal recessive gene conferring susceptibility to lymphosarcoma. We designated this gene ls. Our data

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are compatible with the concepts of genetic susceptibility to lymphosarcoma and of vertical transmission of a virus. Affected rabbits usually died between the age of 5 and 13 months. The neoplastic involvement of lymphoreticular and other organs, especially kidneys, corresponded to a pattern observed in lymphosarcoma of other domestic animals. Specifically, it resembled in many ways visceral lymphosarcomatosis of cats that has been proved unequivocally to be caused by feline leukemia virus. Similarities between rabbit and cat lymphosarcomas include the sites of onset, distribution of the neoplastic lesions, and the finding of a predominantly aleukemic hemogram. However, in rabbit lymphosarcomas, we often found a relative increase in lymphoid cells including both immature and atypical forms (Fox et al., 1970).

If a C-type RNA virus is demonstrated, the ls gene may confer susceptibility to malignant transformation of lymphoreticular tissues. We have found a number of genes in mice that enhance oncogenesis, especially leukemogenesis, but that do not influence the presence or absence of either murine leukemia virus (MuLV) or MuLV antigens. We are now studying the possible viral etiology of rabbit lymphosarcoma. Further, we are investigating possible linkage relationships between ls and other genes in strain WH and related rabbits.

Rabbits are of considerable value in biomedical research because of the vast amount of morphologic, physiologic, and biochemical data available on rabbits, the simplicity of rabbit care and breeding, and their large size. The finding of lymphosarcoma and its hereditary basis provides a new and important model for studies of the pathogenesis of neoplasia. The rabbit colonies at the Jackson Laboratory are free of Shope papilloma and fibroma- and myxomatosis viruses. Except for a small number of epithelial tumors, which we described (Meier et al., 1970, Fox et al., 1971), most tumors in our rabbits have been of lymphoid tissue origin, i.e., lymphosarcoma and thymoma.

(b) Experimental transmission of lymphosarcoma. Cell-free extracts including Moloney concentrates from lymphosarcoma tissue were inoculated by various routes into young adult, newborn, and fetal WH rabbits. No successful transmission has as yet occurred. However, we have successfully transmitted lymphosarcoma with cell suspensions. Whereas young adult WH rabbits are resistant, fetal animals are sensitive to growth of tumor-cell inocula. Newborn rabbits are partially resistant; a single animal developed a tumor at the site of inoculation. In contrast, all four fetuses inoculated with cells at 25 days of pregnancy developed generalized lymphosarcoma. One animal died 4 weeks after birth; the remaining three were killed at 4 weeks of age and revealed typical lymphosarcomas.

(c) Electron microscopic search for complete type C RNA tumor virus(es). Extensive electron microscopic studies of fresh and tissue culture materials from normal and lymphosarcoma-afflicted rabbits of strain WH included the following: thymuses, popliteal and mesenteric lymph nodes, spleens (6 specimens of each), kidneys (5), livers (4), adrenals (2), ovary, testis, and bone marrow (1 each); in addition, we studied fresh liver tissue from seven embryos of 17, 18, and 24 days of fetal age. Tissue cultures consisted of primary, secondary, and established lines (P_3 - P_{20}) from 17- to 24-day whole embryos and lymphosarcoma-affected organs (thymus, spleen, kidney) from adult rabbits. No type C particles were observed, indicating that the viral genome is present in an incomplete or covert form. A single liver sample from a

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lymphosarcomatous rabbit yielded intracisternal (immature type C or A particles?) particles that we do not consider characteristic; no membrane-budding particles could be detected.

The addition to both embryo- and lymphosarcoma-tissue cultures of carcinogenic chemicals has not yet yielded transformed cells; neither has radiation nor iododeoxyuridine (IUdR) and bromodeoxyuridine (BUdR) induced complete infectious type C virus.

(d) Attempts at induction of tumors and virus rescue. In an attempt to induce complete virus formation, we have tried to induce tumors by various means including body irradiation, carcinogens (DMBA, MCA), injections of partially transformed cells, and inoculation of murine type C pseudotype viruses (MSV, RLV, and KiMSV).

The rabbit appears to be extremely resistant to tumor induction by such exogenous means; hence, we are now studying the inducibility, or lack of it, of arylhydrocarbon hydroxylase, an enzyme which converts hydrocarbon compounds to the proximate carcinogen (hydroxylated compounds, epoxides).

Complete infectious virus is not a prerequisite to tumorigenesis; however, isolation of a virus is necessary for the preparation of antisera specific for the rabbit species-specific group-specific antigen (gs-1). In view of our failure so far to induce infectious virus, we have concentrated our efforts on the demonstration of the interspecies-specific group-specific antigen (gs-3) which cross-reacts with specific antisera made to other mammalian type C RNA tumor viruses, e.g., murine and feline viruses.

(e) Search for the interspecies antigen (gs-3) of type C RNA tumor virus. Complement-fixation (CF) tests for the interspecies group-specific antigen (gs-3) of type C RNA tumor virus in strain WH yielded positive results with antiserum MSV I-13 and anticomplementary reactions with goat anti-feline leukemia virus antiserum, IS-8. The two antisera give reactions to the respective viral gs-1 and gs-3 antigens by immunodiffusion; in addition, each antiserum reveals an unidentified line probably due to a viral coat- or tissue antigen. Since rabbit tissues cannot react with either this antigen or the species-specific gs-1, the CF reaction obtained with MSV I-13 must be specific for gs-3.

Similarly, we found, by indirect peroxidase-labeling, specific gs-3 reactivity of several tissue culture rabbit tumor lines. Gs-3 reactions were also obtained upon immunodiffusion from both normal and tumorous tissues including the thymus, lymph nodes, gastrointestinal tract, and gonads. The immunodiffusion studies were done in collaboration with Drs. Stephen Oroszlan and Raymond V. Gilden, Flow Laboratories, Inc. Studies are now in progress utilizing the radioimmune (precipitation) assay (RIPA or RIA) developed by Dr. Wade Parks (Viral Carcinogenesis Branch, National Cancer Institute) and Dr. Gilden, respectively, and designed to specifically reveal gs-3; this test is the most refined and is several fold more sensitive than both the direct CF- and immunodiffusion tests. Generally, precipitation of less than 90% in the RIPA is considered positive. Thus, unless an anticomplementary reaction is occurring, several of the samples tested are reproducibly positive including skeletal muscle. In order to definitively rule out a nonspecific effect, assays of

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additional samples, including normal rabbit tissues are anticipated.

(f) Assays and distribution of DNA-polymerases in rabbit tissues. RNA tumor virus(es) contain an enzyme, RNA-dependent DNA polymerase, that transcribes DNA from an RNA template. The role of this enzyme has not as yet been established, i.e., whether or not it plays a part in transformation or may be essential for a transformed state.

In collaboration with Drs. Masa Hatanaka and R. V. Gilden, Flow Laboratories, we assayed organs of normal, azathioprine (immuran)-treated and lymphosarcoma-afflicted rabbits. Most organs, both normal and malignant, revealed enzyme activity. Thymuses, major mesenteric and popliteal lymph nodes, and gastrointestinal tract have the highest specific activity of RNA-dependent DNA polymerase suggesting the common nature or origin of the lymphopoietic systems. Also, the data taken together with our serological findings verify the presence of an RNA viral genome in WH rabbits. It is also present in other incipient inbred strains of rabbits, i.e., IIIDw and a hybrid between two strains (III_{mo}/III_c).

(g) Pedigree analyses for lymphosarcoma- and immune hemolytic anemia susceptibilities. We have now observed 75 cases of lymphosarcoma in strain WH and seven cases in genetically related rabbits of strain AX. Autoimmune hemolytic anemia occurred in 60 rabbits of strain X; in addition, four cases were found in strain AC which is in part derived from strain X. In fact, all affected individuals in the four strains are genetically related and trace back to a common ancestor, X974. Thus, we suspect that the two different syndromes, each caused by an autosomal recessive gene, ls and ha, respectively, may indeed be manifestations of the same gene with the phenotype dependent upon the remainder of the host genotype. To either confirm or rule out this possibility, we are now awaiting results of sufficient matings between ls/+ and ha/+ heterozygotes.

Certain of these aspects including our pathological and hematological findings in strain WH and X afflicted with lymphosarcoma and immune hemolytic anemia have been presented at the First Annual Symposium of Hycel, Inc. (Fox, 1972) and in two book chapters (Fox, 1973; Lindsey and Fox, 1973).

(h) Myeloid leukemia in strain III. We have now found the first case of myeloid leukemia in the rabbit. It occurred in a 13-1/2 month old male of subline III_{ep}. Its features are distinct from hereditary lymphosarcoma by cell type, organ involvement, and distribution of tumors. Studies are in progress to determine whether this case of myeloid leukemia is of hereditary origin. Also, we plan to determine whether an oncogenic type C RNA viral genome is involved in this, as well as in lymphosarcoma of WH rabbits (Meier et al., 1972).

(i) Genetic predisposition to tumors in the rabbit. Our analysis of genetic factors has been presented in Progress Report 2, and a paper describing our findings has been published (Fox et al., 1971).

(2) Hereditary hemolytic anemia associated with thymoma in strain X rabbits. Autoimmune (Coombs-positive) hemolytic anemia occurs with high frequency in rabbits of strain X. This condition is rapidly fatal with a mean sur-

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vival time of about 5 months. Sometimes it is associated with thymic hyperplasia and thymoma. The gene conferring susceptibility, designated ha, may be identical with that causing lymphosarcoma susceptibility and assigned the gene symbol, ls, in WH rabbits. Strain X and WH are closely related genetically, and a common gene responsible for all conditions may have phenotypic expressions that are dependent upon the remainder of the genotype. We are also considering the possibility that a vertically transmitted virus similar or analogous to the C-type RNA virus of mice is of etiological importance in addition to gene(s) conferring susceptibility (Fox et al., 1971).

Methods and procedures of proposed studies. In our studies of the pathogenesis of lymphosarcoma in WH rabbits and of hemolytic anemia associated with thymoma in strain X rabbits, we wish to achieve an understanding of the underlying mechanisms leading to each disorder in the two strains. No doubt the interactions of host genotype, "environment," and C-type RNA virus, if present, are complex. In the light of our findings to date we are proposing the following studies:

(1) Studies in strain WH rabbits. Apparently complete infectious C-type RNA tumor is absent or occurs only rarely. Screening of tissues from lymphosarcoma-affected, normal, and embryonic rabbits by electron microscopy has not as yet yielded C-type particles. Thus, we are no longer attempting cell-free transmission of lymphosarcoma.

(a) If complete virus is indeed absent, we may (1) induce complete viral synthesis by carcinogenic chemicals, immunosuppressive agents, and various nucleosides, and (2) continue to screen rabbit tissues for the cross-reacting (gs-3) viral group-specific antigen using at least two antisera that can detect the interspecies determinant by gel diffusion.

(b) If complete virus is inducible in any strain of rabbits, its isolation and purification is essential for the production of specific antisera.

Approaches a2 and b have been described in previous applications. In the past year, several new ways and means of tumor-induction in rabbits have evolved that may bring us closer to the successful activation of the putative endogenous C-type viral genome. These are generally relevant to cocarcinogenesis and relate to the following observations and procedures:

(a) Inducibility of aryl hydrocarbon hydroxylase in rabbits. Aryl hydrocarbon hydroxylase (AHH) is representative of a class of oxidative microsomal enzyme systems that hydroxylates a variety of compounds including carcinogenic polycyclic hydrocarbons. A number of pertinent observations now support the view that the metabolism of such carcinogens is related to both their induced carcinogenicity and cytotoxicity. The inducibility of AHH is strain-dependent in mice and hamsters; in fact, it depends on a single autosomal gene. Strains that develop high basal AHH activity in response to treatment with polycyclic hydrocarbons would be expected to produce high levels of reactive intermediates with enhanced carcinogenicity. Although we do not have as yet rigorous data on the inheritance of AHH inducibility in rabbits, various strains differ greatly in their inducibility. Strain III is regularly inducible and thus is an excellent strain for chemical carcinogenesis studies.

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(b) Transplacental carcinogenesis by 1-ethyl-1-nitrosourea. We have found in mice that susceptibility or resistance to 1-ethyl-1-nitrosourea (ENU) is clearly hereditary; these findings are of considerable importance regarding the proposed "direct action" of ENU and, possibly, other nitrosocompounds. It now appears that the outcome of ENU-induced effects most likely depends upon genetically determined differences in the cellular repair mechanism rather than in its metabolism or transplacental uptake, although we found a certain association with the inheritance of AHH inducibility.

Developing embryos are particularly susceptible to ENU, and an inverse relationship exists between its teratogenicity and carcinogenicity.. Strain III_{VO} fetuses are highly susceptible to ENU-induced renal tumors when pregnant mothers are treated on day 18 of pregnancy; multiple tubular adenomas and adenocarcinomas develop as early as 40 days after birth. These tumors occur reproducibly and within a limited time, and provide us with an opportunity to search for endogenous rabbit C-type tumor virus(es).

(c) Intratracheal application of 3-methylcholanthrene. We are injecting 3-methylcholanthrene (MCA) intratracheally into both AHH-inducible and non-inducible strains of rabbits. MCA is suspended in a sterile medium of physiological saline and 0.2% gelatin (w/v) to give a concentration of 50 mg MCA/0.5 ml medium per injection. Several injection schedules are used. If presently available animal models are to be effectively applied to studies on the etiology and pathogenesis of human lung cancer, detailed information on the histogenesis of experimentally induced respiratory tract tumors is essential. The tumors that may develop will again be screened for C-type viruses.

(d) Potentiation of methylcholanthrene-carcinogenicity by tobacco condensate. Aqueous extracts of cigarette-smoke condensates have been found to enhance benzpyrene-induced liver AHH and menadione reductase activities in rats. Without benzpyrene, AHH was moderately increased by tobacco condensate, whereas menadione reductase was unaffected. Since we found most strains of rabbits extremely resistant to MCA carcinogenesis, we are administering to various strains of rabbits aqueous extracts of cigarette-smoke condensates in sugared drinking water. After 1 month they are then injected with MCA in trioctanoin. Tobacco condensates are kindly provided by Dr. A. R. Patel of Meloy Laboratories, Springfield, Va., and through the courtesy of Dr. John Kreisher, The Council for Tobacco Research, USA--Inc.

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Thus, we aim by various means to induce tumors of both epithelial (pulmonary, renal) and mesenchymal (sarcomatous) types in efforts to simultaneously activate endogenous C-type virus. Radiation-leukemia virus (RadLV) of C57BL/6J mice exemplifies the induction of complete virus in a strain of mouse that ordinarily lacks infectious virus expression. Another example is the induction of the group-specific antigen in negative strains, e.g., C57L/J mice by carcinogenic chemicals; clearly no genetic information for antigen-synthesis is conferred by the chemical.

(2) Studies in strain X rabbits. Information is needed to decide whether (1) the same gene that is responsible for susceptibility to hereditary immune hemolytic anemia also predisposes to thymoma, (2) the gene giving rise to hemolytic anemia or thymoma, or both, in strain X is the same gene that is responsible for lymphosarcoma-susceptibility in WH rabbits, and (3) both hemo-

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lytic anemia and thymoma are due to an interaction with a vertically transmitted C-type RNA viral genome.

The following procedures should provide the answers sought to the first two questions. Information on the third will result from approaches identical or analogous to those described for WH rabbits.

(a) Is the same gene responsible for both hemolytic anemia and thymomagenesis? We are chronically treating strain X rabbits homozygous for the hemolytic anemia trait (ha/ha) with the immunosuppressive drug, azathioprine (Imuran). Since the hemolytic disease is due to an immune disorder, azathioprine should prevent its occurrence. However, by analogy to NZB mice, it should not interfere with development of thymoma. Thus, in order to establish that the gene is responsible for both conditions, we must be able to selectively induce thymoma upon continued azathioprine therapy.

(b) Are the genes, lymphosarcoma-susceptibility (ls) and hemolytic anemia (ha) identical? In order to answer the question of allelism of ha and ls, F_1-hybrids between respective heterozygotes (ls/+ and ha/+) should yield approximately 25% "abnormal" offspring if the two genes are allelic. We cannot of course decide a priori whether they resemble either of the respective homozygotes (ls/ls and ha/ha) or whether they have clinical features of both.

Genetic studies are time-consuming and require large numbers of animals. Also, we cannot know what the latency period may be for clinical signs to appear in case compound heterozygotes (ls/ha) are obtained.

In past and current breeding experiments we have made the following crosses, ha/ha x ls/+ and ha/+ x ls/+; indeed, several compound heterozygotes have appeared. These findings clearly indicate the identity of ha and ls. In order to obtain a representative spectrum of phenotypic expression of compound heterozygotes, we anticipate a requirement of at least 10 afflicted rabbits.

So far, affected rabbits suffered either from hemolytic anemia or a combination of hemolytic anemia and a lymphoproliferative disorder depending upon the age at which clinical signs appeared.

(3) Both strains of rabbits. Strains WH and X are "partially inbred." The coefficient of inbreeding, which defines that proportion of loci for which the original or base population was heterozygous but which through inbreeding has become homozygous, is approximately 0.7 and 0.8, respectively (Appendix). We estimate, therefore, that each strain may be homozygous for as many as 75% of the initially variable loci and that these loci have been fixed for different alleles in the two strains because of deliberate selection for and maintenance of specific mutant genes.

A population of animals need not be inbred for an analysis of the inheritance of a specific gene or phenotypic trait. The genetic basis of a trait by which two strains differ can be obtained by crosses to obtain F_1-, F_2-, and backcross generations. From populations segregating in Mendelian proportions for recognizable phenotypes, one may estimate the number of gene pairs that

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distinguish the two parental strains with respect to the trait in question. Conversely, because there is reduced genetic variability within inbred strains, they are unsuitable for selection experiments unless heterogenous "foundation" populations are synthesized by crossing two or more strains. Since the two rabbit strains are now only "partially inbred," we can select for those genes that produce a particular phenotype of interest, e.g., lymphosarcoma susceptibility and resistance. Thus, in each strain we should be able to produce two or more lines that may vary in tumor development and also a line that is tumor-resistant. These may interact specifically, but variously in studies of chemical or other cocarcinogenesis.

In addition to the 75 cases of lymphosarcoma in strain WH, we have now also found seven cases in genetically related rabbits of strain AX. Also, in addition to the 60 cases of autoimmune hemolytic anemia in strain X, four cases were observed in strain AC, which is in part derived from strain X. In fact, all affected individuals in the four strains are genetically related and trace back to a common ancestor, X974.

We are now looking for circulating antinuclear and anti-DNA antibodies in the various rabbit strains, as well as immune complex disease in biopsies from kidneys. Since lymphoid cell lines maintained in suspension on a gyratory shaker have yielded complete virus from NZB and related mice, we are using analogous conditions for cell lines produced from spleens and lymph nodes.

Significance of this research. The proposed studies relate to an opportunity for the analysis of two major groups of disorders: (1) cancer and (2) autoimmune disease. They do not deal directly with the effects of tobacco, but are clearly relevant to tobacco effects in several indirect ways: (1) neither tobacco or its various chemically-defined components nor all known carcinogenic chemicals provide a host with the genetic information to produce or induce cancer and any other disease, (2) the occurrence of cancer or any non-neoplastic disease is dependent upon the inborn host-genetic regulation of all processes allowing or disallowing it to occur. Thus, an analysis of the hereditary pathways and their acquired modifications through tobacco or other means is fundamental to an understanding of all disease processes. Some of these may be attacked by use and study of the two (ls/ls and ha/ha) mutants of rabbits. Because of their analogies, they may help clarify mechanisms of the respective human disorders and provide basic information about their pathogenesis.

So far, we have made three major findings: (1) the likely identity of the genes conferring susceptibility to both cancer (lymphosarcoma) and immune hemolytic anemia (autoimmune disease), (2) the most probable presence in strains WH, X, and other strains of an endogenous oncogenic C-type RNA tumor viral genome, and (3) strains WH and X have Coombs' autoantibodies. The major significance of our studies lies in their potential relationship:

(1) Hereditary autoimmune disease in strain X. Like human systemic lupus erythematosus (SLE) and the related disease in New Zealand black (NZB) mice and (NZB x NZW) F_1 hybrid mice, hereditary autoimmune disease in strain X is associated with synthesis of various autoantibodies to erythrocytes and various nuclear materials. Some of the possibilities for their occurrence are that: (1) the hosts are unable to properly repair nucleic acids, and altered nucleic acids might be released from cells into the circulation. Such altered nucleic acids might either have an abnormal catabolic fate or antigenicity. This situation would be analogous to the defect in the repair of DNA that occurs in xeroderma pigmentosum. (2) The hosts are deficient in or have abnormal

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nucleases, and perhaps other catabolic enzymes, that result in the formation of immunogenic breakdown products from spent, autologous cells. (3) The hosts have a defect in some intracellular structure such as the nuclear or plasma membrane resulting in an inability to keep nucleic acids in a proper configuration or an intracellular compartment. And (4), the hosts harbor a virus whose genome is immunogenic because of a different configuration or nucleotide composition than that of the host, thereby terminating tolerance to autologous nucleic acids.

Thus, it seems possible that cells from patients with SLE or diseased NZB mice are variants of one or more of the defects listed. Their detection and evaluation is clearly important in elucidating the pathogenesis of SLE, as well as the catabolism, structure, and repair of nucleic acids in normal cells.

(2) Hereditary lymphosarcoma in strain WH. In addition to autoantibodies to double-helical DNA, DNA-histone complexes, single-stranded DNA, and nucleolar RNA and their complexes, NZB mice also make antibodies to the endogenous C-type RNA tumor virus. The presence of such a genome in both strains WH and X, as well as all other rabbit strains, is most probable. Thus, with genes ls and ha being identical and conferring susceptibility to either lymphosarcoma or autoimmune hemolytic anemia, a common pathogenesis of the two disorders is likely.

We now have evidence for a highly significant association in mice between the endogenous C-type RNA viral genome and tumorigenesis. In fact viral expression in early life is a highly predictable marker for tumorigenesis with advancing age. This expression is host-gene controlled, and relates to tumors of all types, i.e., mesenchymal as well as epithelial tumors, and leukemias as well as solid tumors. Thus, the mechanism for tumorigenesis is hereditary or "built-in," but whether or not tumors will develop depends upon other host-genes as well as environmental factors, be they tobacco or other potential cocarcinogens. The histological type of the target cell, e.g., alveolar, epidermoid, lymphoid, fibroblastic, etc., represents the chief determinant of the pathological kind of tumor that is finally induced. Although this explanation requires ultimate substantiation, it provides the most rational basis for all available tumor data.

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Biographical sketches of all principal and professional personnel.

Hans Meier

Born:	21 July 1929, Zurich, Switzerland	
Nationality:	United States citizen	
Education:	University of Zurich (Veterinary Medicine), D.V.M.	1954
	University of Zurich (Pathology) Dr. med. vet.	1957
	Harvard School of Public Health	1957
	Massachusetts Institute of Technology, Alumnus (Biophysical and biochemical cytology, biology)	1959
Honors:	Royal Society of Health (Pharmacology), M.R.S.H.	1963
	Member, Scientific Advisory Board, The Council for Tobacco Research, USA	1971
	Member, Solid Tumor-Virus Working Group, Special Virus-Cancer Program, National Cancer Institute, NIH	1971
Major Interests:	Experimental pathology, cancer	

Positions:

1970-	The Jackson Laboratory, Senior Staff Scientist
1970-	The National Cancer Institute, Consultant
1962-70	The Jackson Laboratory, Staff Scientist
1960-62	The Jackson Laboratory, Associate Staff Scientist
1957-60	Harvard Medical School, Res. Asst. (Pathology)
1958-60	Children's Hospital and Medical Center, Boston, Asst. (Experimental) Pathologist

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	1957-60	Children's Cancer Research Foundation Res. Asst., Res. Assoc. (Exp. Pathol.)
	1954-57	Angell Memorial Hospital, Boston, Fellow, Resident, Chief of Pathology
Postgraduate training:	1955-57	Harvard Medical School, Course work in pathology and neuro-pathology
	1957	Harvard University, Summer School of Arts and Sciences, and of Education (Biophysics and radioactive measurements)
	1958	Armed Forces Institute of Pathology (Course in histochemistry)
	1959	Harvard University, Depts. Biology, (Electron microscopy) and Chemistry (Experimental methods in biochem.)

Societies: N. Y. Acad. Sci.; Soc. Exp. Pathol.; Int. Acad. Pathol.; Amer. Soc. Pathol. and Bacteriol.; Cancer Soc.; Histochem. Soc.; Amer. Soc. for Cell Biol.; New England Soc. Pathol.; A.A.A.S.; Conference Res. Workers in Animal Diseases; Basic Research Council, Amer. Heart Ass.; Amer. Ass. Public Health; Royal Society of Health (M.R.S.H.); Soc. Exp. Biol. Med.

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Richard R. Fox

Born:	12 November 1934, New Haven, Connecticut
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Educated:	University of Connecticut (Animal Production) B.S. 1956
	University of Minnesota (Animal Breeding) M.S. 1958
	University of Minnesota (Animal Breeding and Genetics) Ph.D. 1959
	The Jackson Laboratory (Rabbit Genetics), Post-doctoral Fellow 1959-60
Honors:	B.S., with honors and with distinction in Animal Production
	Sigma Xi
	Gamma Sigma Delta
	Alpha Zeta
	Member: Committee on Nomenclature for Inbred Rats, Hamsters, Gerbils, and Rabbits, Nat. Acad. Sci., ILAR
Major interests:	Genetics of the rabbit; quantitative genetics; reproductive physiology; animal husbandry
Positions:	1965- The Jackson Laboratory, Staff Scientist
	1961- University of Maine, Department of Animal and Veterinary Science, Lecturer
	1960-65 The Jackson Laboratory, Assoc. Staff Scientist
Societies:	Amer. Genet. Soc.; Genet. Soc. of Amer.; Amer. Dairy Sci. Ass.; Amer. Ass. Lab. Anim. Sci.; Amer. Soc. Anim. Sci.; Teratology; A.A.A.S.

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Source: https://www.industrydocuments.ucsf.edu/docs/yfyd0078

Budget justification

The studies proposed in this application take advantage of several strains of rabbits and mutant stocks available at the Jackson Laboratory. These may prove of considerable value in studies of tobacco products.

Although a wide variety of spontaneous infectious and hereditary diseases have been found in the rabbit, tumors have been reported infrequently. However, only a few systematic studies have been conducted. Our studies reveal that lymphosarcomas and hemolytic anemia occur with high frequency in rabbits, but that the incidence, type, and development are greatly influenced by age, breed, and other constitutional factors. Clearly, studies with genetically controlled rabbits both supplement and complement studies with inbred mice. This unique resource of rabbits at the Jackson Laboratory must be maintained and made available to research workers elsewhere. The two strains of rabbits, WH and X, are extremely valuable for studies in oncogenesis but their exploitation has hardly begun. Strain III is an excellent strain because of its AHH-inducibility. Fortunately, we have the professional staff and talent essential to the studies that we propose. The financial support requested from the Council for Tobacco Research for maintenance and study of these rabbits is minimal, but is adequate when coupled with existing support. New stainless steel cages are required for replacement of worn-out galvanized cages.

There is no need, at present, to include in this budget salary provision for 100% effort contributed by the Project Co-Directors, because this proposal relates to work supported by NIH research contract PH43-67-744 from the National Cancer Institute and NIH resource grant RR 00251 from the Division of Research Resources of NIH.

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Source: https://www.industrydocuments.ucsf.edu/docs/[illegible]

TABLE 1: CHARACTERISTICS OF RABBIT STRAINS AT THE JACKSON LABORATORY AS OF 15 OCTOBER 1972.

STRAIN (Name & Symbol)	COLOR	PREDOMINANT GENOTYPE	ORIGIN	COEFFICIENT OF INBREEDING(1)	PREDOMINANT ALLOTYPE	MATURE WEIGHT (g)	OTHER GENES SEGREGATING
III (sublines)	Albino	cc AA E^dE^d	New Zealand White Castle '32	0.97	$a^1a^1b^4b^4c^7c^7$	3800	1, scoliosis(2)
(III mo)				(0.97)			
(III vo)		(rcrc)		(0.93)			
(III ep)		(ep/ep)		(0.96)			
(III ah)		(Ah/ah)		(0.93)			
(III wu)		(Wu/wu)		(0.90)			
(III Da)		(Da/da)		(0.90)			
(III Dw)		(Dw/dw)		(0.95)			
(III vp)		(Vp/vp)		(0.92)			
(III cd)		(Cd/cd)		(0.96)			
III_c	Albino	cc AA E^dE^d mpmp	Subline of III	0.90	$a^3a^3b^4b^4c^7c^7$	4000	
X	Sooty yellow	$C(c^{ch2})$ aa ee bb Hahа	Castle's small race	0.82	$a^3a^3b^4b^4c^7c^7$	2200	dk, ±21-, As
WH	Agouti	AA EE WHWH Lsls(5)	Rockefeller Inst. 1949. Crossed with strains X, III, etc.	0.72		2300	e, r^2
AC(3)	Black, recessive white marking	aa E^dE^d, du^du^d Acac(5), Sbsb(5)	Dutch	0.76	$a^3a^3b^5b^5c^7c^7$	2400	y, du^w, c^{7-}
ACEP(3)	Black, recessive white marking	aa E^dE^d VV du^w/du^w, epep	Dutch	0.92	$a^3a^3b^4b^4c^{7-}c^{7-}$	2400	hg, c, v
ACCR (B)(3)(6)	Albino	cc sasa	Dutch	Inbred	$a^3a^3b^5b^5c^7c^7$	2400	y_{7+}
(Y) (6)	Albino	cc sasa	Dutch	Inbred	$a^3a^3b^4b^4c^{7-}c^{7-}$	2400	c^{7+}
OS	Black, minimal recessive white marking	aa E^dE^d, du^du^d, Osos(5)	Dutch Rockefeller Institute 1948	0.84	$a^2a^2b^4b^4c^7c^7$	3200	hydrocephaly(2), du^w
AX	Chinchilla	$c^{ch3}c^{ch3}$ AA ww Axax(5)	Outcross of Chinchilla race V to strains III and X	0.68	$a^3a^3b^4b^4$	3500	du, bu, c^{ch2}, c; hypospadias
AX_{bubu}	Albino	cc bubu	AX strain	0.76	$b^4b^4c^7c^7$	3500	c^{ch3}, c^{ch2}, a^1a^3
A	Albino	cc	Miscellaneous	0.93	$a^3a^3b^4b^4$	2700	1
C	agouti or black	AA or aa	Miscellaneous	0.96		2800	b, c, du

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TABLE 2: GENES OF IMPORTANCE TO STUDIES OF CONSTITUTIONAL DISEASE BEING MAINTAINED IN THE RABBIT COLONY AT [illegible]

Disease and Gene Symbol	Disease and Gene Symbol	Disease and Gene Symbol
Achondroplasia (ac)	angora (l)	ataxia (ax)
dach (Da) (6)	furless (f)	buphthalmia (bu)
dwarf (Dw) (4)	rex^2 (r^2)	epilepsy (audiogenic seizures, ep)
renal cysts (rc)	satin (sa)	gamma globulin alleles, Aa^1, As^2, Aa^2
mandibular prognathism (mp)	adrenal hyperplasia (ah)	gamma globulin alleles, Ab^4, Ab^5
hypogonadia (hg)	chondrodystrophy (cd)	gamma globulin alleles, Ac^7, Ac^{7-}
lymphosarcoma (ls)		osteopetrosis (os)
hemolytic anemia (ha)		spina bifida (sb)

Lethal muscle contracture, hypognathia, splay leg, diminutive dwarf, and cleft palate occur sporadically in some strains and may be genetic.

For other gene symbols, see:

Sawin, P. B. 1955. Recent genetics of the domestic rabbit. Adv. Genet. 7:183-226.
Robinson, R. 1958. Genetic studies of the rabbit. Bibliog. Genet. 17:229-558.
Fox, R. R. 1972. Taxonomy and Genetics In Biology of The Laboratory Rabbit. S.W. Weisbroth, A.L. Kraus, R.E. Flatt (eds) Academic Press (In Press).

Footnotes:

(1) Maximum coefficient in the strain computed according to Wright's formula for coefficient of inbreeding (F). Inbreeding is by sib mating or as close to sib mating as possible consonant with maintenance of the specific lethal or semi-lethal genes (indicated by underlining) and an optimal reproductive capacity and viability.

(2) Level of penetrance is dependant upon environmental conditions.

(3) Sublines of the same Dutch stock obtained from Rockefeller Institute in 1948.

(4) Formerly symbolized dw, then recognized in the heterozygote (Sawin 1955 Adv. Genet. 7:183) and the symbol changed to Dw to reflect a semidominant.

(5) The ac, Da, os, sb, ha, ls, ah, cd, and ax genes are maintained in their respective strains by progeny testing of prospective parents. Homozygous transmitters are obtainable from the same test.

(6) The ACCR (B) and ACCR (Y) strains are also referred to simply as strain B or Y respectively.

(7) Formerly da, now recognized, by ear papilla, in the heterozygote.

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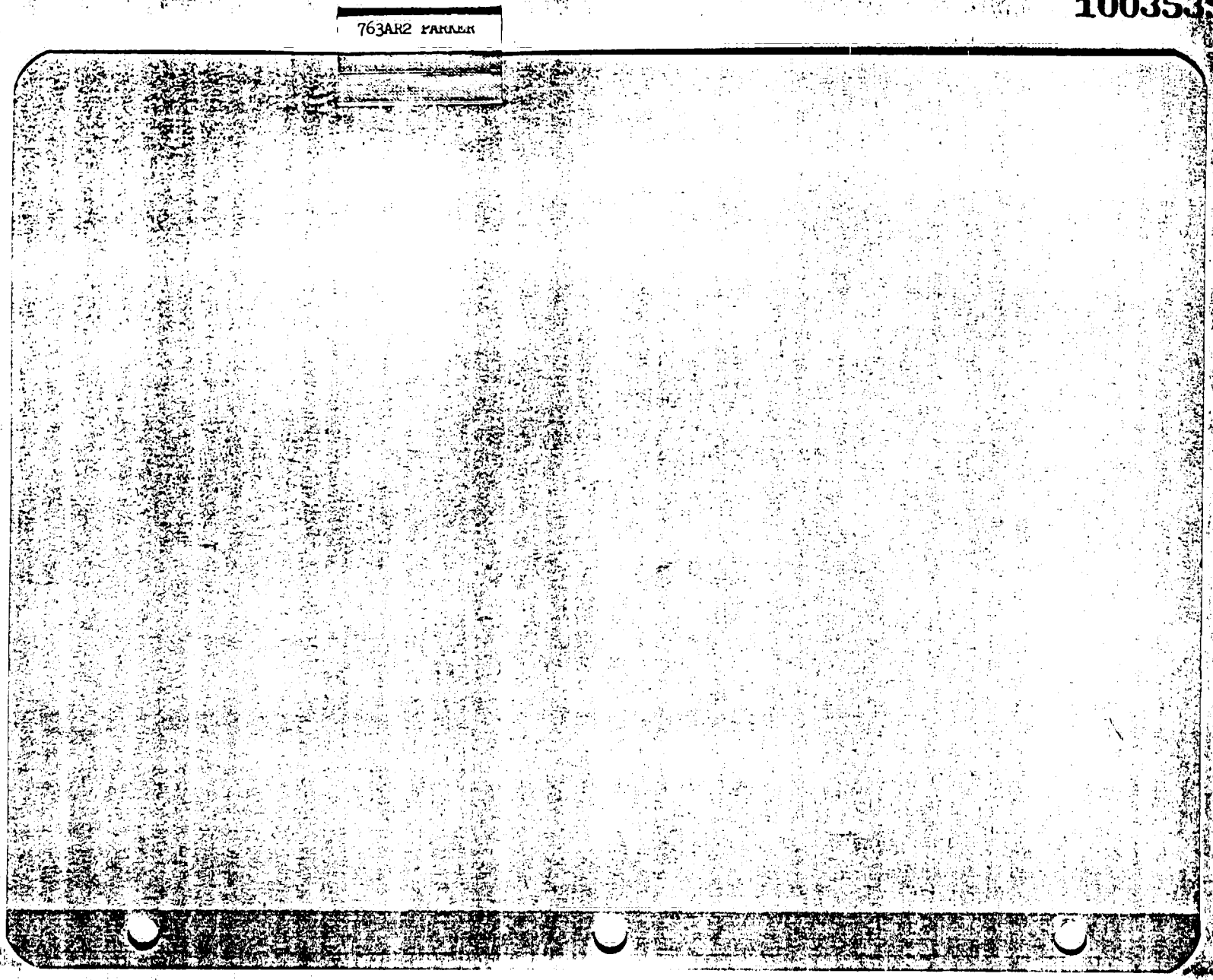

Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

THE COUNCIL FOR TOBACCO RESEARCH–U.S.A., INC.

P-5

February 5, 1973

Grant Application No. 763AR2

To: The committee comprising Drs. Andervont, Gardner, Huebner

Subject: John W. Parker, M.D., University of Southern California School of Medicine, Los Angeles
Renewal application No. 763AR2
"Immune Suppression by Cigarette Smoke and Chemical Carcinogens"

History

CTR support of this study began in 1970.

This request is for $37,950 for the third and final year of a three year program. The current grant is $29,890.

Documents Submitted (attached)

1. Application dated January 29, 1973.

2. Progress report 1/25/72 to 1/25/73.

3. Manuscript "Periodate Induced Lymphocyte Transformation . . .", Parker, O'Brien, Steiner and Paolilli; in press "Experimental Cell Research".

(The remaining publications mentioned on the last page of the application are available if you so request.)

Comment

Dr. Richard L. O'Brien of this group presented "Lymphocyte Transformation Induced by Sodium Periodate" at our Macrophage Conference November 30 and December 1, 1972.

F.W.N.

FWN:wg
Encls.

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Source: http://www.industrydocuments.ucsf.edu/docs/yyy0068

Comm.
Dr. Andervont
Dr. Gardner
Dr. Huebner

CARCINOGENESIS

#763AR2
#763AR1-7/1/72-6/30/73
#763A-7/1/71-6/30/72
#763-7/1/70-6/30/71

THE COUNCIL FOR TOBACCO RESEARCH—U.S.A., INC.
110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8585

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Application for Research Grant
(Use extra pages as needed)

Date: January 29, 1973

1. Principal investigator (give title and degrees):

John W. Parker, M.D. — Associate Professor of Pathology
Richard L. O'Brien, M.D. — Associate Professor of Pathology

2. Institution & address:

Department of Pathology
University of Southern California School of Medicine
2025 Zonal Avenue
Los Angeles, California 90033

3. Department(s) where research will be done or collaboration provided:

Department of Pathology

4. Short title of study:

"Immune Suppression by Cigarette Smoke and Chemical Carcinogens"

5. Proposed starting date: July 1, 1973

6. Estimated time to complete: Two years

7. Brief description of specific research aims:

We wish to determine whether cigarette smoke inhalation or the parenteral administration of chemical carcinogens, some of which are components of cigarette smoke, suppress cellular immunity when measured by in vitro assays of lymphocyte activation. In brief, we shall determine the effects of cigarette smoking and of chemical carcinogens on the ability of mouse lymphocytes to undergo blastic transformation in response to the non-specific mitogens phytohemagglutinin (PHA), pokeweed mitogen (PWM) and Na IO_4, and in a mixed leukocyte culture (MLC) assay.

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8. Brief statement of working hypothesis:

It has been postulated for sometime and by a number of investigators that one of the mechanisms involved in oncogenesis is the suppression of the immune response of the host so that its surveillance against spontaneously arising or induced neoplastic cells is faulty. In the face of this faulty surveillance system the neoplastic cells may establish themselves to the extent that the immune mechanism is no longer capable of eliminating them. It has been shown in the past that chemical carcinogens can suppress humoral and cellular immunity. The hypothesis that we are testing is that cigarette smoke or purified chemical carcinogens may suppress cellular immunity and that this suppression can be measured by in vitro functions of lymphocytes. The importance of testing this hypothesis is based on the premises that lymphocytes are important mediators of cellular immunity and that the in vitro assays of lymphocyte transformation reflect lymphocyte function in vivo.

9. Details of experimental design and procedures (append extra pages as necessary)

Background:

There is a good deal of information in the literature which suggests that the destruction of neoplastic cells in vivo and in vitro involves the direct attack of sensitized lymphocytes on target neoplastic cells. In this respect it resembles transplantation rejection phenomena and involves the cellular immune system in neoplastic cell destruction. It has been postulated that deficiencies in the cellular immune system may allow either induced or spontaneously arising neoplastic cells to survive and establish themselves as tumors. These considerations have led us to undertake studies attempting to determine the effects of carcinogens on the functional integrity of the cellular immune system.

There are several ways to assay the integrity of cellular immunity. That which we use is the measurement of in vitro responses of lymphocytes to blastogenic and mitogenic stimuli. It has been demonstrated in the past that chemical carcinogens, particularly polycyclic hydrocarbons, are capable of suppressing the cellular and humoral immune responses. These studies have utilized for the most part in vivo assays. These are useful for measuring in vivo functional integrity but are limited in their ability to provide an opportunity to study cellular and subcellular mechanisms by which immune suppression occurs. For this purpose the in vitro assays of lymphocyte transformation in response to blastogenic stimuli are advantageous. Our laboratories utilize the response of lymphocytes to PHA, PWM, Na IO_4 and MLC by blastic transformation. These in vitro assays of lymphocyte functional integrity are useful because they are simple, highly reproducible and not time consuming. Further, the MLC assay represents the ability of the lymphocytes to recognize as abnormal or foreign the surface of other cells. It is expected that it is this recognition which enables lymphocytes to destroy neoplastic cells when they arise in vivo. The validity of these in vitro assays as a measure of in vivo function of the lymphocytes is supported by the findings of a number of investigators that the depression of lymphocyte responsiveness to PHA and other mitogens in vitro is correlated with clinically evident immunologic depression when it occurs either as a component of a disease state or when induced with immunosuppressive agents.

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For the last two and a half years the Council for Tobacco Research has supported our studies of the effects of carcinogenic chemicals on these in vitro lymphocyte functions. We have measured the effects of chemicals administered to lymphocytes in vitro and given in vivo to experimental animals. To the present time, we have found that the chemical carcinogens dimethylbenz-anthracene (DMBA) and 4-nitroquinoline-N-oxide (4-NQO), when administered to the cells in vitro, inhibit their responses to PHA, PWM and MLC. This inhibition occurs at low concentrations in the neighborhood of 10^{-7} to 10^{-6}M. We have also found that non-carcinogenic but related compounds, specifically pyrene and benzanthracene, do not inhibit these reactions when administered in vitro. The effects of in vitro administration of hydrocarbons is not perfectly correlated with the carcinogenicity of the compounds used. For example, we have also found that methylcholanthrene (MCA), a potent carcinogen, does not inhibit the response of cells to PHA or PWM. It does inhibit transformation in MLC but the inhibition requires higher concentrations of MCA than of DMBA and 4-NQO.

We have also conducted studies attempting to measure the effect of these carcinogens when administered in vivo. We have injected MCA subcutaneously into C57 black mice. At varying times after exposure to the hydrocarbon, we have sacrificed the mice and measured the ability of their cells to respond to PHA and MLC stimulation. At the present time the results are preliminary but they indicate that the administration of the carcinogenic compound suppresses the ability of lymphocytes to respond to the in vitro mitogenic stimuli. This is not so in the control group. Interestingly, these decreases precede and coincide with the appearance of tumors in the animals treated with carcinogen. The preliminary nature of the experiments done so far is due to the fact that the mitogenic assays on our mice have been complicated by apparent exogenous antigenic stimulation of the lymphocytes so that in all our assays we have a rather high background of lymphocyte transformation. Consequently the amount of suppression of in vitro induced transformation by carcinogens is difficult to quantitate. We have dealt with this technical problem by doing our experiments on specific pathogen-free mice kept in isolation to diminish exogenous antigen stimulation. These measures and exploring various proportions of stimulator and responder cells in MLC have resolved the technical problem and our assays now have very low background transformation. Thus over the next four to five months we will be gathering data which will allow us to quantitate quite confidently the effect of carcinogens on the ability of the lymphocytes to respond to the in vitro mitogenic stimuli provided by PHA, PWM and MLC.

We wish to extend these studies to a larger group of carcinogenic chemicals, including other classes of carcinogenic compounds. We wish also to extend them to determine the effects of chronic cigarette smoking on these in vitro measures of lymphocyte functional integrity. We have already done one assay of in vitro transformation of lymphocytes from smoke exposed mice in Dr. Clayton G. Loosli's laboratory. The results were inconclusive because our control mice were a different strain of C57 black mice, a great deal younger and had been breathing ambient Los Angeles air, but they were sufficiently interesting to stimulate our desire to conduct a carefully controlled study.

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During the period of support by the Council for Tobacco Research we have also developed and explored another assay for in vitro measurement of lymphocyte function. This is a system for the transformation of lymphocytes by a short treatment with Na IO_4. We have published several papers on this transformation (see accompanying progress note) and we wish now to include it as an in vitro measure of lymphocyte functional integrity in the mice which we are challenging with chemical carcinogens and with chronic cigarette smoking. In general terms it is similar to the in vitro transformation stimulated by PWM and PHA, but it is sufficiently different, particularly with reference to the cell population stimulated, that we feel it is an important addition to our studies.

Experimental Design:

To measure the effect of cigarette smoking on lymphocyte responses to the in vitro mitogenic stimuli we will use to a large extent the same mice that Dr. Clayton G. Loosli is using in his studies of the effects of cigarette smoke on the lungs of mice.

C57 black, CD1 and Snell mice (pathogen-free) will be exposed to smoke from three standard low nicotine cigarettes in a single exposure per day in a CTR-Walton horizontal smoke exposure machine. Control mice will spend an equal time in the smoke exposure machine but they will be breathing filtered air. Control and smoke exposed mice will be housed in separate but identically constructed filtered air rooms. Groups of mice exposed to smoke of three cigarettes per day will be sacrificed at three month intervals and the responses of their lymphocytes to PHA, PWM, Na IO_4 and MLC will be measured. Groups of mice will also be removed from smoke exposure at varying times and their lymphocytes will be studied at intervals thereafter to determine whether any changes observed during the smoking period are progressive, static or reversible. All experiments on lymphocyte transformation will be correlated with Dr. Loosli's studies on the pulmonary lesions occurring in these mice. In all experiments, an appropriate number of non-smoking control mice of the same age as the experimental animals will be sacrificed and their lymphocytes studied in the same manner.

The design for studying the effects of purified carcinogens on in vitro lymphocyte transformation will be similar to that which we are following now. We will select compounds from the benzacridine, the nitrosamine and the polycyclic hydrocarbon classes of chemical carcinogens and structurally related non-carcinogenic analogs for test. The chemical carcinogens and control compounds will be dissolved in trioctanoin or other suitable solvents and injected subcutaneously into mice, either in single doses or as a cumulative dose spaced over a period of several weeks. At varying times thereafter they will be sacrificed and the responses of their lymphocytes to PWM, PHA, Na IO_4 and MLC will be assayed.

The experimental animals will be specific pathogen-free C57 black mice which will be kept in an isolated environment to prevent their contacting exogenous infectious agents and other antigenic stimuli. Stimulator cells for use in the MLC will be derived from spleens of CBA mice and treated with mitomycin-C to prevent their blastic transformation in the culture system. All of these methods are currently in use in our laboratories.

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

10. Space and facilities available (when elsewhere than item 2 indicates, state location):

Facilities currently available for this research are the combined research spaces of Drs. O'Brien and Parker. This includes 3,000 sq.ft. of research space in the Hoffman Research Building plus 1,000 sq.ft. of communally shared research space in the same building. This program has available to it the facilities and technical expertise necessary for a wide variety of experimental procedures. These include tissue culture, biochemical and biophysical procedures and electron microscopy.

A list of the major equipment available for this research in our laboratories includes three electron microscopes, a Coulter counter and cell size plotter, a Zeiss photomicroscope, several light, phase and dissecting microscopes, Beckman models L4 and L3-50 ultracentrifuges, a Sorvall refrigerated high speed centrifuge, two refrigerated International low speed centrifuges, two liquid scintillation counters, spectrophotometers, a spectrophotofluorometer, incubators for cell cultures, and darkroom facilities. We also have available to us the facilities of the University of Southern California School of Medicine Vivarium for the procurement and custody and care of experimental animals.

11. Additional facilities required:

None.

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12. Biographical sketches of investigator(s) and other professional personnel (append): Appended.

13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available). Appended.

Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

14. First year budget:

A. Salaries (give names or state "to be recruited")		% time	Amount
Professional (give % time of investigator(s) even if no salary requested)			
John W. Parker, M.D.	Investigator	40% *	-
Richard L. O'Brien, M.D.	Investigator	10%	-

REDACTED

Technical			
Joan Steiner, M.D.	Research Assistant	100%	
Jo Ann Vukmanovich	Laboratory Assistant	100%	
	Fringe Benefits, 10%		

REDACTED

Sub-Total for A

B. Consumable supplies (by major categories)

Tissue culture supplies	3,000
Chemicals	2,000
Animals, purchase and care	6,000
	11,000

Sub-Total for B

C. Other expenses (itemize)

Travel - One national meeting for each investigator	900
Publication costs	200
Sub-Total for C	1,100
Running Total of A + B + C	33,000

D. Permanent equipment (itemize)

None

Sub-Total for D

E. Indirect costs (15% of A+B+C) E 4,950

Total request 37,950

15. Estimated future requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	R	12,000	1,100	none	5,257	40,302

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

16. Other sources of financial support:
List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Mechanisms of Suppression of Cellular Immunity by Carcinogenic Hydrocarbons	Council for Tobacco Research-USA, Inc. #763AR1	29,890	7-1-72 to 6-30-73
Mechanisms of Environmental Carcinogenesis	John A. Hartford Foundation, Inc.	161,266	5-1-72 to 4-30-74
Special Fellowship for a sabbatical year at the Walter & Eliza Hall Institute of Medical Research, Melbourne, Australia (J.W. Parker)	National Cancer Institute, NIH #1-FO3-CA-52894-01	14,000 (stipend) + travel	9-1-72 to 8-31-73

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Lymphocyte Transformation by Periodate	Department of Health, Education & Welfare, NIH	88,865	7-1-73 to 6-30-76 (3 yrs)

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal Investigator John W. Parker, M.D.
Typed Name Richard L. O'Brien, M.D.
Signature Richard L O'Brien John W Parker Date 1-29-73
Telephone 213 (Area Code) 225-1511 (Number) 392 or 387 (Extension)

Check's payable to
University of Southern California
c/o Mr. Clark A. McCartney, Director,
Mailing address for checks Dept. Contracts & Grants
University of Southern California
University Park, Los Angeles, Calif. 90007

Responsible officer of institution
Typed Name Mr. Z. A. Kaprielian, Vice President,
Title Academic Administration and Research
Signature [signature] Date JAN 31 1973
Telephone 213 225-1511 432

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R: REDACTED MATERIAL

BIOGRAPHICAL SKETCH — JOHN W. PARKER, M.D.

Name:	John W. Parker, M.D.
Academic Title:	Associate Professor of Pathology
Date of Birth:	REDACTED
Citizenship:	
Sex:	Male
Marital Status:	REDACTED

EDUCATION:

Institution and location	Degree	Year	Scientific Field
University of Arizona, Tucson, Arizona	REDACTED		Zoology
Harvard Medical School, Boston, Mass			Medicine

HONORS:

Phi Beta Kappa, Phi Kappa Phi, Phelps Dodge Scholarship	University of Arizona	1949-1953
Marshall Foundation Scholarship,	Harvard Medical School	1953-1959
Dernham Sr. Fellow in Oncology,	Am. Cancer Soc. (Calif)	1964-1969

RESEARCH AND PROFESSIONAL EXPERIENCE:

1972-1973	Visiting Sr. Research Fellow, Walter & Eliza Hall Institute of Medical Research, Melbourne, Australia (Sponsor: Dr. Donald Metcalf, Head, Cancer Research)
1968-present	Associate Professor of Pathology, University of Southern California School of Medicine, Los Angeles.
1969-present	Head, Electron Microscopy Section, Department of Pathology, University of Southern California School of Medicine, Los Angeles.
1971-present	Research Consultant, Professional Staff Association, Los Angeles County-U.S.C. Medical Center.
1964-1968	Assistant Professor of Pathology, U.S.C. School of Medicine, Los Angeles.
1964-1969	Dernham Sr. Fellow in Oncology, American Cancer Society (Calif. Div.) - Research in tumor immunology.

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1962-1964	Clinical Instructor in Pathology, University of California School of Medicine, San Francisco.
1962-1964	Chief, Clinical Laboratories, Veterans Administration Hospital, San Francisco.
1958-1962	Resident in Pathology, University of California Medical Center program, part-time research during this period.
1957-1958	Intern (straight medicine), University of California Medical Center, San Francisco.

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

PUBLICATIONS

1. Parker, J.W., Grodsky, G.M. and Carbone, J.W. A study of the metabolites of florantyrone. Gastroenterology, 41:563, 1961.
2. Parker, J.W., Elevitch, F.R. and Grodsky, G.M. Binding of fluorescent insulin to intracellular antibodies in guinea pigs immunized with insulin. Proc. Soc. Exp. Biol. Med., 113:48, 1963.
3. Tavel, F.R., Osina, T.G., Parker, J.W., Goodfriend, R.B., McGonigle, D.J., Jassie, M.P., Simmons, E.L., Tobenkin, M.D., and Schule, J.W. Retroperitoneal lymph node dissection: A morbid study. J. Urol., 89:241, 1963.
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7. Lukes, R.J., Parker, J.W., Bell, R.E., McBride, N.L., and Madill, K.R. Canine lymphomas histologically indistinguishable from Burkitt's lymphoma. Lancet, 2:389, 1966.
8. Parker, J.W., Wakasa, H., and Lukes, R.J. Cytoplasmic fibrils in mixed lymphocyte cultures. Blood, 29:608, 1967.
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12. Wakasa, H., Parker, J.W., and Lukes, R.J. Cytoplasmic filamentous structures in activated lymphocytes. An electron microscopic study. In Proceedings of the Third Annual Leucocyte Culture Conference, ed. W.C. Rieke, Appleton-Century-Crofts, 1969, pp. 573-585.
13. Parker, J.W., and Lukes, R.J. The variability of PHA-induced lymphocyte transformation in repetitive studies. In Proceedings of the Third Annual Leucocyte Culture Conference, ed. W.C. Rieke, Appleton-Century-Crofts, 1969, pp. 111-128.
14. Parker, J.W., Steiner, J., Coffin, A., Lukes, R.J., Burr, K., and Brilliantine, L. Blastomitogenic agents in Leguminosae and other families. Experientia, 25: 187, 1969.
15. Parker, J.W., and Lukes, R.J. Spontaneous lymphocyte transformation following Hong Kong flu. In Proceedings of the Fourth Annual Leucocyte Culture Conference, ed. O.R. McIntyre, Appleton-Century-Crofts, 1971, pp. 281-298.
16. Lukes, R.J., Tindle, B.H., and Parker, J.W. Reed-Sternberg-like cells in infectious mononucleosis. Lancet, 2:1003, 1969.
17. D'Ablaing, G., Rogers, E.R., Parker, J.W., and Lukes, R.J. A simplified and modified methyl green pyronin stain. Am. J. Clin. Path., 54:667-669, 1970.
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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

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20. Jacobs, A., O'Brien, R.L., Parker, J.W. and Paolilli, P. DNA repair following exposure of human lymphocytes to 4-nitroquinoline-N-oxide. Internat. J. Cancer 10:118, 1972.

21. Parker, J.W., O'Brien, R.L. and Lukes, R.J. Transformation of human lymphocytes by sodium periodate. Lancet 1:103-104, 1972.

22. Tindle, B.H., Parker, J.W. and Lukes, R.J. "Reed-Sternberg cells" in infectious mononucleosis? (A report of eight cases.) Am. J. Clin. Path. 58: 607-617, 1972.

23. Parker, J.W., O'Brien, R.L., Lukes, R.J., Steiner, J. and Paolilli, P. Periodate-induced lymphocyte transformation. 1. Inhibition by sulfhydryl reducing agents and sugars. Immunol. Comm. 1(3):263-277, 1972.

24. Puffer, H.W., Parker, J.W., Russell, F.E. and Warner, N.E. Pathology of Loxosceles laeta venom poisoning. In Proceedings of the Internat. Soc. of Toxicol. (Presented Sept. 10-15, 1972, Darmstadt, West Germany). In press.

25. Jacobs, A.J., O'Brien, R.L., Parker, J.W. and Paolilli, P. Abnormal DNA repair of 4-nitroquinoline-1-oxide induced damage by lymphocytes in xeroderma pigmentosum. Mutation Research 16:420-424, 1972.

26. Parker, J.W., O'Brien, R.L., Steiner, J. and Paolilli, P. Periodate induced lymphocyte transformation. 2. Character of response and comparison with phytohemagglutinin and pokeweed mitogen stimulation. Exptl. Cell Res. - (Accepted for publication)

27. Parker, J.W., O'Brien, R.L. and Steiner, J. Periodate induced lymphocyte transformation. 3. In preparation.

28. Brown, I., Nies, K. and Parker, J.W. Surface immunoglobulins and mitogenic response of lymphocytes from patients with chronic lymphocytic leukemia. In preparation.

29. Parker, J.W. and O'Brien, R.L. Comparative morphology of lymphocytes transformed by periodate, phytohemagglutinin and pokeweed mitogens. An electron microscopic study. In preparation.

30. Russell, F.E., Chin, D. and Parker, J.W. Anatomy of the Tarantula venom gland. In preparation.

31. Parker, J.W. and Streng, C. Lymphocyte transformation in steroid deficient mice. In preparation.

32. Russell, F.E. and Parker, J.W. Ophthalmic pathology produced by cobra venom. In preparation.

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BOOK Lukes, R.J. and Parker, J.W. Disorders of Hematopoiesis, Chapter 25, in Concepts of Disease, ed. Brunson, J. and Gall, E. MacMillan Co., New York, 1971, pp. 881-954.

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ABSTRACTS

1. Parker, J.W., Wakasa, H. and Lukes, R.J. Defective lymphocyte transformation in Hodgkin's disease. Sixth Internat. Congress of the Internat. Acad. Path., Kyoto, Japan, Oct. 13-19, 1968.
2. Lukes, R.J. and Parker, J.W. A critical re-evaluation of lymphocyte transformation in Hodgkin's disease. Sixty-Sixth Annual Meeting of the Am. Assn. Path. Bact., 1969, San Francisco. Am. J. Path. 55:22a, 1969.
3. Gardner, M.B., Parker, J.W. and Lukes, R.J. Histoicgic and ultrastructural observations of the feline Burkitt-like lymphoma. Sixty-Sixth Annual Meeting of the Am. Assn. Path. Bact., 1969, San Francisco. Am. J. Path. 55:26a, 1969.
4. Smith, W.C., Kaneshiro, M.M., Goldstein, B.D., Parker, J.W. and Lukes, R.J. Gaucher cells in chronic granulocytic leukemia. Annual Meeting of the Internat. Acad. Path., 1969, San Francisco. Lab. Invest 20:603, 1969.
5. Parker, J.W., O'Brien, R.L., Lukes, R.J. and Steiner, J. Inhibition of sodium periodate induced lymphocyte transformation by reducing agents. Fed. Proc. 31:796, 1972.
6. Jacobs, A.J., O'Brien, R.L., Parker, J.W. and Paolilli, P. Repair of 4-nitroquinoline-N-oxide induced damage by normal lymphocytes and those from xeroderma pigmentosum. Fed. Proc. 31:625, 1972.
7. Puffer, H.W., Russell, F.E., Parker, J.W. and Warner, N.E. Pathology of Loxosceles laeta venom poisoning. (Abstract) Internat. Soc Toxicol., Sept. 10-15, 1972 (Darmstadt, West Germany).

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R: REDACTED MATERIAL

BIOGRAPHICAL SKETCH — RICHARD L. O'BRIEN, M.D.

Name:	Richard L. O'Brien, M.D.
Academic Title:	Associate Professor of Pathology
Date of Birth:	
Place of Birth:	REDACTED
Citizenship:	
Sex:	Male
Marital Status:	REDACTED

EDUCATION:

Institution and Location	Degree	Year	Scientific field
Creighton University, Omaha, Neb.	REDACTED		Physiology
Creighton University, Omaha, Neb.			Medicine

HONORS:

Post-sophomore Research Fellowship, USPHS, 1957-1958
Alpha Omega Alpha, 1959
White House Fellowship Finalist, 1966
Special Fellow, National Cancer Institute, 1967-1969

RESEARCH AND PROFESSIONAL EXPERIENCE:

1970-present:	Associate Professor of Pathology, University of Southern California School of Medicine, Los Angeles.
August 1966-present:	Research on the mechanisms of chemical and viral oncogenesis and control of chromosome replication.
1966-1970:	Assistant Professor of Pathology, University of Southern California School of Medicine, Los Angeles.
1964-1965:	Capt. M.C., Department of Molecular Biology, Walter Reed Institute of Research, Washington, D.C. Research on the interaction of anti-malarial compounds with DNA, the effect of these interactions on the synthesis of nucleic acids, and the relationship of these interactions to the biologic activity of the antimalarials.
1962-1964:	Postdoctoral fellow in biochemistry, Enzyme Institute, University of Wisconsin, Madison. Research on energy transduction in mitochondria and movement of substances across biological membranes.

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1960-1962	Intern and Assistant Resident (Medicine), 1st Medical Division, Bellevue Hospital, New York, N.Y.
1957-1958	Postsophomore research fellow of USPHS, Creighton University School of Medicine, Omaha, Nebraska. Research on the relationships between the reticuloendothelial system and the action of cortisone and STH; research on the relationships between the sympathetic nervous system and blood pressure control.
June and July 1957:	Research fellow of the Allergy Foundation of America, Veterans Administration Hospital, Omaha, Nebraska. Research of serotonin and histamine in sensitized guinea pig uteri.

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Richard L. O'Brien, M.D.

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2. O'Brien, R. L., Mc Carthy, H. H., and Wilhelmj, C. M.: Effect of R. E. blockade on cortisone and STH activity. Physiologist, 1:57, 1958.

3. Wilhelmj, C. M., O'Brien, R. L., and Mc Carthy, H. H.: Sympathetic imbalance and blood pressure homeostasis. Physiologist, 1:86, 1958.

4. O'Brien, R. L., and Koszewski, B. J.: Humoral factors in myeloproliferative disorders. Clin. Res., 7:285, 1959.

5. O'Brien, R. L., Luna, M., Pettinger, W., Mc Carthy, H. H., and Wilhelmj, C. M.: Reticuloendothelial blockade and capillary resistance response to cortisone and STH. Proc. Soc. Exp. Biol. Med., 100:264, 1959.

6. O'Brien, R. L., and Brierley, G. P.: Penetration of heart mitochondria by nucleotides, Pi and other ions. Fed. Proc., 23:266, 1964.

7. Brierley, G. P., Murer, E., and O'brien, R. L.: Studies on ion transport. VI. The accumulation of Mg ++ by heart mitochondria in the absence of inorganic phosphate. Biochim. Biophys. Acta, 88:650, 1964.

8. George, J. N., O'Brien, R. L., Pollack, S., and Crosby, W. H.: Glucose enhancement of RBC membrane damage by primaquine. Clin. Res., 13:273, 1965.

9. Allison, J. L., O'Brien, R. L., Hahn, F. E.: DNA: Reaction with chloroquine. Science, 149:1411, 1965.

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13. O'Brien, R. L., and Hahn, F. E.: Chloroquine: Structural requirements for binding to DNA and antimalarial activity. Antimicrob. Agents Chemother., 315, 1965.

14. George, J. N., O'Brien, R. L., Pollack, S., and Crosby, W. H.: Studies of <u>in vitro</u> primaquine hemolysis: Substrate requirement for erythrocyte membrane damage. J. Clin. Invest., 45:1280, 1966.

15. O'Brien, R. L., Allison, J. L., and Hahn, F. E.: Nature of the DNA-chloroquine complex. Fed. Proc., 25:558, 1966.

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17. O'Brien, R. L., Olenick, J. G., and Hahn, F. E.: Reactions of quinine, chloroquine and quinacrine with DNA and their effects on the DNA and RNA polymerase reactions. Proc. Nat. Acad. Sci., 55:1511, 1966.

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19. O'Brien, R. L., Allison, J. L., and Hahn, F. E.: Evidence for intercalation of chloroquine into DNA. Biochim. Biophys. Acta, 129:622, 1966.

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21. Tai, H. T., and O'Brien, R. L. Multiplicity of viral genomes in an SV40 transformed hamster cell line. Virology 38:4;698-701, 1969.

22. Tai, H. T., and O'Brien, R. L. Multiplicity of viral genomes in SV40 transformed cells. Am. J. Path., 55:7a, 1969 (Presented at AAPB Meeting, 1969).

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25. Parker, J. W., O'Brien, R. L., Lukes, R. J., and Steiner, J. Transformation of human lymphocyte's by sodium periodate. Lancet 2:103-104, 1972.

26. O'Brien, R. L., Sanyal, A. B., and Stanton, R. H. Association of DNA replication with the nuclear membrane in HeLa cells. Expt. Cell Res. 70:106-112, 1972.

27. Jacobs, Allan J., Richard L. O'Brien, John W. Parker & Patricia Paolilli. DNA repair following exposure of human lymphocytes to 4-nitroquinoline-1-oxide. Internat. J. Cancer, 10:118, 1972.

28. Parker, J. W., O'Brien, R. L., Lukes, R. J. and Steiner, J.: Inhibition of sodium periodate induced lymphocyte transformation by reducing agents. Fed. Proc. 31:796, 1972.

29. Jacobs, A. J., O'Brien, R. L., Parker, J. W. and Paolilli, P.: Repair of 4-nitroquinoline-N-oxide induced damage by normal lymphocytes and those from xeroderma pigmentosum. Fed. Proc. 31:625, 1972.

30. Parker, J. W., O'Brien, R. L., Lukes, R. J., Steiner, J. and Paolilli, P.: Periodate-induced lymphocyte transformation. I. Inhibition by sulfhydryl reducing agents and sugars. Immunol. Comm. 1(3):263-277, 1972.

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31. Jacobs, A.J., O'Brien, R.L., Parker, J.W. and Paolilli, P.
Abnormal DNA repair of 4-nitroquinoline-1-oxide induced damage by lymphocytes in xeroderma pigmentosum. Mutat. Res. 16:420-424, 1972.

32. Parker, J.W., O'Brien, R.L., Steiner, J. and Paolilli, P.
Periodate induced lymphocyte transformation. 2. Character of response and comparison with phytohemagglutinin and pokeweed mitogen stimulation.
Exptl. Cell Res. (Accepted for publication)

33. Parker, J.W., O'Brien, R.L. and Steiner, J.
Periodate induced lymphocyte transformation. 3.

34. Parker, J.W. and O'Brien, R.L.
Comparative morphology of lymphocyte transformed by periodate, phytohemagglutinin and pokeweed mitogen. An electron microscopic study.
(In preparation)

35. O'Brien, R.L.: Sites of chromosome replication in HeLa cells.
Submitted to Exptl. Cell Res.

36. Craig, P. and O'Brien, R.L.
Phytohemagglutinin (PHA)-responsive histone kinase activity in human peripheral lymphocyte nuclei.
Fed. Proc. (Abstract) - In press.

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Recent Pertinent Publications*:

1. Parker, J.W., O'Brien, R.L., Lukes, R.J. and Steiner, J.
Transformation of human lymphocytes by sodium periodate.
Lancet 1:103-104, 1972.

2. Jacobs, A.J., O'Brien, R.L., Parker, J.W. and Paolilli, P.
DNA repair following exposure of human lymphocytes to 4-nitroquinoline-1-oxide.
Internat. J. Cancer 10:118-127, 1972.

3. Parker, J.W., O'Brien, R.L., Lukes, R.J., Steiner, J. and Paolilli, P.
Periodate induced lymphocyte transformation. 1. Inhibition by sulfhydryl reducing agents and sugars.
Immunol. Comm. 1:263-277, 1972.

4. Parker, J.W., O'Brien, R.L., Steiner, J. and Paolilli, P.
Periodate induced lymphocyte transformation. 2. Character of response and comparison with phytohemagglutinin and pokeweed mitogen stimulation.
Exptl. Cell Res. (Accepted for publication.)

5. Jacobs, A.J., O'Brien, R.L., Parker, J.W. and Paolilli, P.
Abnormal DNA repair of 4-nitroquinoline-1-oxide induced damage by lymphocytes in xeroderma pigmentosum.
Mutat. Res. 16:420-424, 1972.

In preparation:

Parker, J.W., O'Brien, R.L. and Steiner, J.
Periodate induced lymphocyte transformation. 3.

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* Reprints and pre-print enclosed.

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

Council for Tobacco Research Grant #763A
Investigators: John W. Parker, M.D.
Richard L. O'Brien, M.D.

PROGRESS REPORT #4
Period: 1-25-72 to 1-25-73

Title of Study:

"Mechanisms of Suppression of Cellular Immunity by Carcinogenic Hydrocarbons"

The effect of carcinogenic hydrocarbons in vivo and in vitro on cellular immunity:

The studies described in the previous progress report indicated that spleen cells from mice injected with methylcholanthrene (MCA) showed progressively less response in a one-way mixed lymphocyte reaction (MLR) and a suggestion of less response to phytohemagglutinin (PHA). This depression becomes most marked at the time when tumors were apparent at the site of injection. A factor that complicated the study was that some of the mice, particularly in the carcinogen-injected groups, developed infections because of local necrosis and this produced variability in spleen cell responses. It also raised questions as to the validity of assuming that it was the carcinogen that was immunosuppressive. For this reason we have purchased pathogen-free mice and are in the process of repeating the experiments, keeping the animals in quarters isolated from all other experimental animals. This experiment with groups of animals injected with MCA, dimethylbenzanthracene (DMBA), anthracene and solvent is in progress. By using pathogen-free mice, treating stimulator cells with mitomycin C and titrating the relative proportions of stimulator and responder cells in culture, we have resolved the technical problems encountered before and now anticipate no further difficulties. While procuring the animals and establishing optimal conditions for these experiments, we have studied the effect of these agents on lymphocyte transformation when they are added directly to the cultures.

It can be seen in Figs. 1, 2 and 3 that DMBA suppresses the response to PHA and PWM, but that MCA and anthracene do not. Fig. 4 shows that the potent carcinogen 4-nitroquinoline-N-oxide (4-NQO) also suppresses the response to PHA and PWM and it is even more potent than DMBA in this regard. We have also found that DMBA and 4-NQO when administered to the cells in vitro inhibit the mixed leukocyte culture transformation. The MLC is even more sensitive to these inhibitory effects than are the responses to PHA and PWM. In contrast to its lack of inhibition of the PHA and PWM induced transformation, MCA inhibits the MLC transformation. It is, however, effective only at concentrations significantly higher than the inhibitory concentrations of DMBA and 4-NQO. Benzanthracene fails to inhibit the MLC transformation. These studies have measured lymphocyte transformation by the incorporation of tritiated thymidine into DNA. Thus, in reality we are measuring the ability of these compounds to inhibit DNA synthesis by the lymphocytes. This could be the result either of inhibition of transformation, a direct inhibition of DNA synthesis or possibly even cell killing.

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The viability of cells in treated and untreated cultures was essentially the same, indicating that the depression of lymphocyte response was due to a lack of tranformation or inhibition of DNA synthesis rather than cell death. We have not yet answered this latter question but studies aimed at answering it are continuing.

Related studies:

Out interests in the broadest sense are in the control mechanisms involved in cellular proliferation and specifically how they are altered by carcinogens and in neoplastic transformation. This applies not only to the target cells for carcinogenic activity, but to the non-neoplastic model of reversible transformation and proliferation, i.e. lymphocyte transformation. This model provides an excellent means of studying the interrelationships between cell membranes, cellular control mechanisms, and proliferation and for determining the effects of carcinogenic agents on these interrelationships. It is fortuitous that the lymphocyte is the major mediator of the cellular immune response to neoplasms. Therefore studies utilizing this model have implications far exceeding what might appear at first glance.

Since it is impossible to separate and isolate such studies, we have, in addition to the studies initially proposed, been utilizing and developing an attractive lymphocyte transformation model which involves stimulation of lymphocytes with a compound that has specific chemical actions, i.e., periodate (IO_4^-). IO_4^- ion triggers lymphocytes to transform after only a few seconds of exposure and then is no longer required. It can be washed away after stimulation and is also a specific oxidant of vicinal hydroxyl groups which are found in the carbohydrates. Therefore it offers an excellent model for studying the nature of the triggering event, the interrelationships between the cell surface where the triggering event apparently occurs and the genetic control mechanisms in the nucleus. Further, it offers another in vitro assay of functional lymphocyte integrity. In addition, the effect of carcinogens on these events and the abnormalities that exist in neoplastic lymphocytes in this response can be studied. We have gained some information about IO_4^- stimulation of human lymphocytes and have presented it in several publications which are listed and of which copies are included. This work has been supported in part by (and acknowledged) the Council for Tobacco Research-U.S.A.

Finally, we have studied the effect of the carcinogen 4-NQO on DNA repair in normal human lymphocytes and those from patients with xeroderma pigmentosum. These studies have also been reported and reprints are included.

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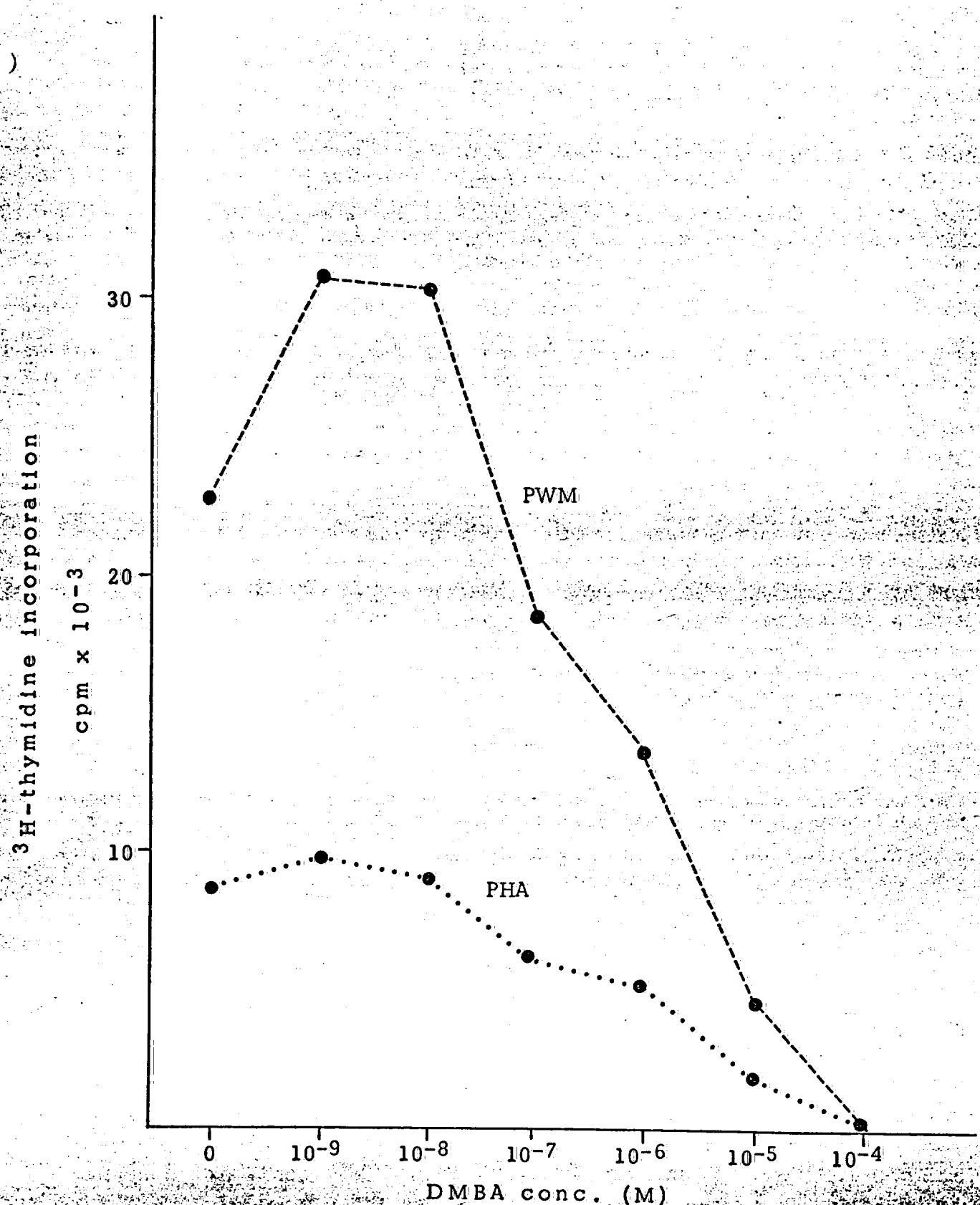

Fig. 1

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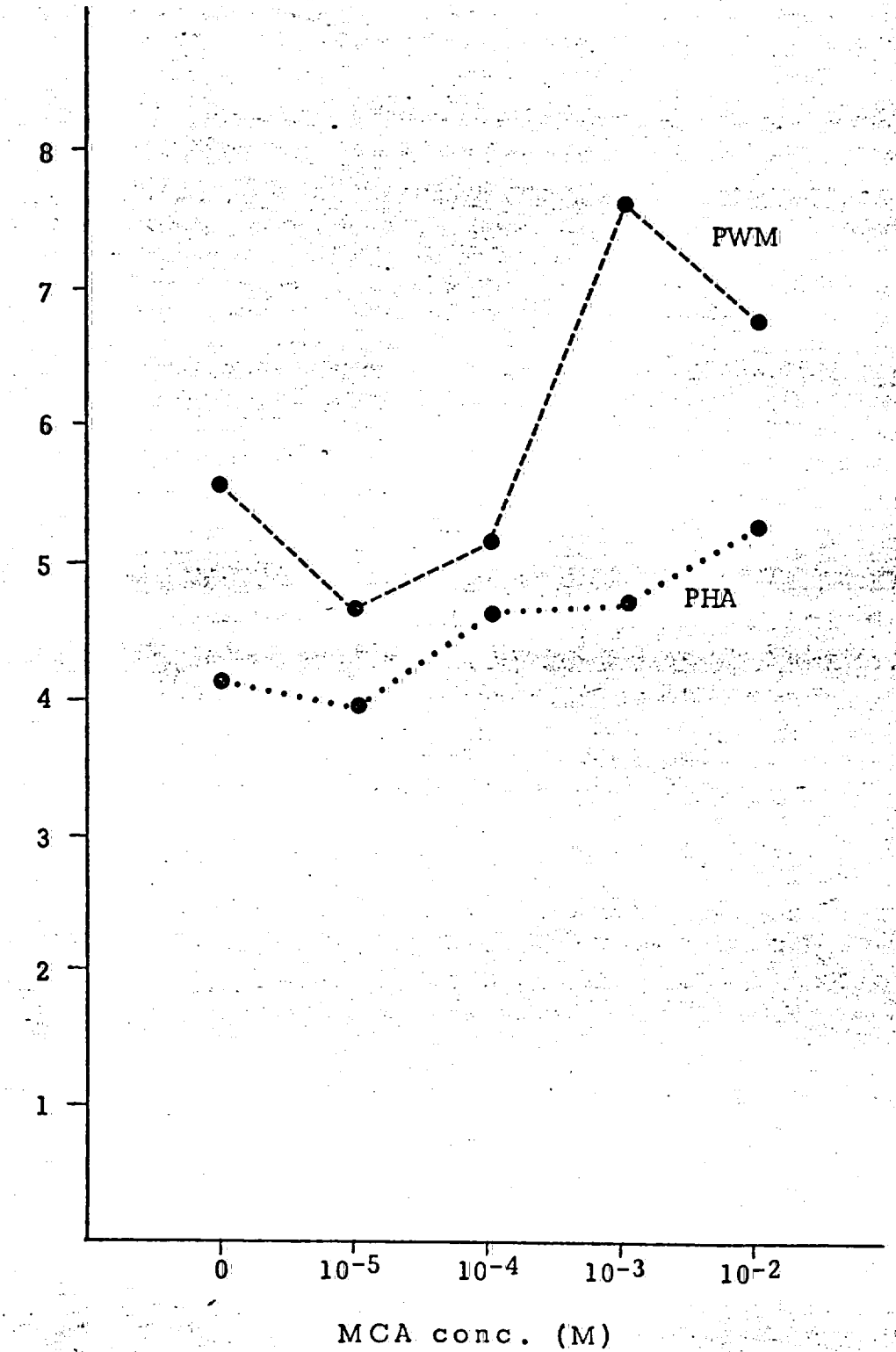

Fig. 2

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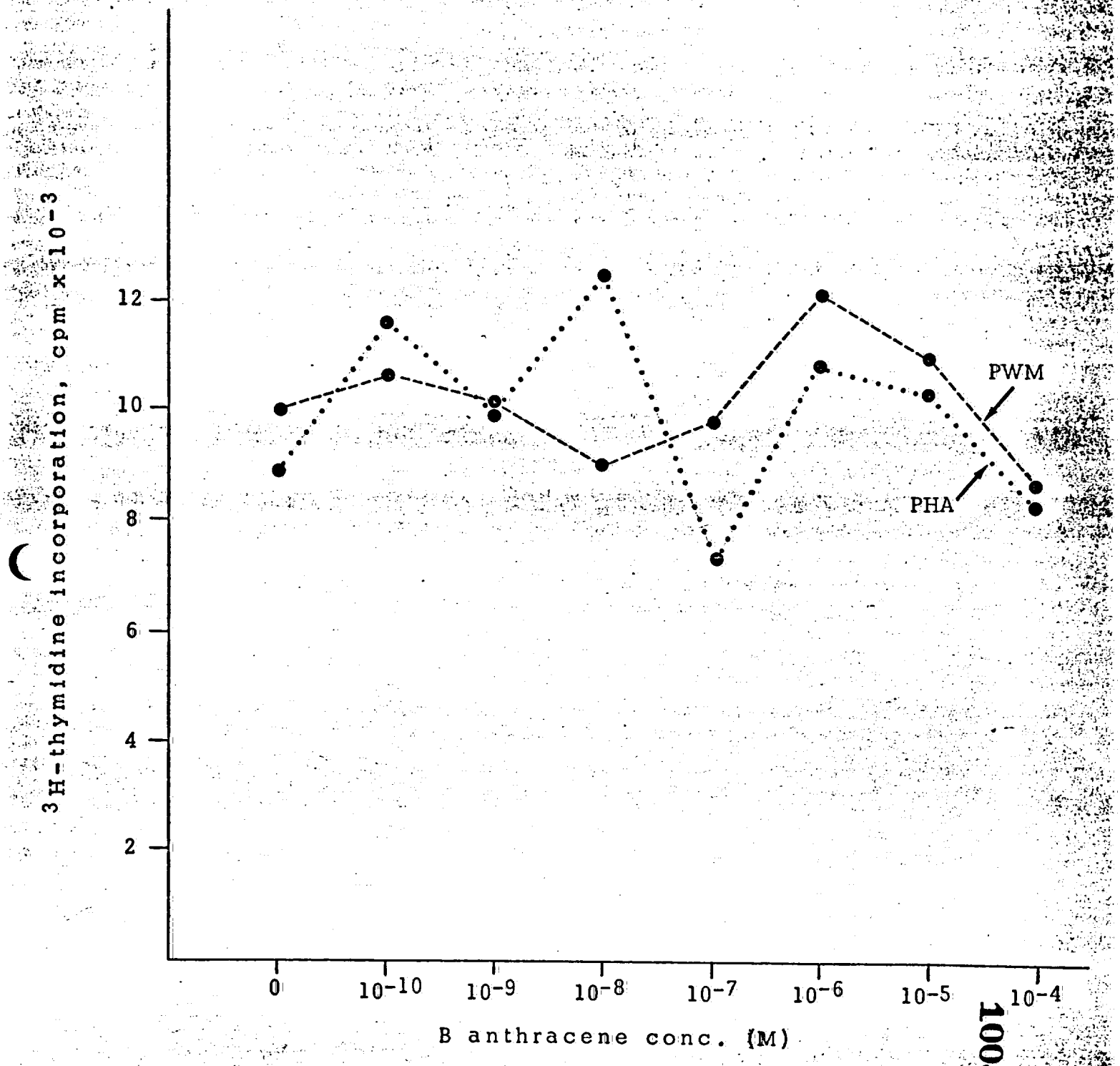

Fig. 3

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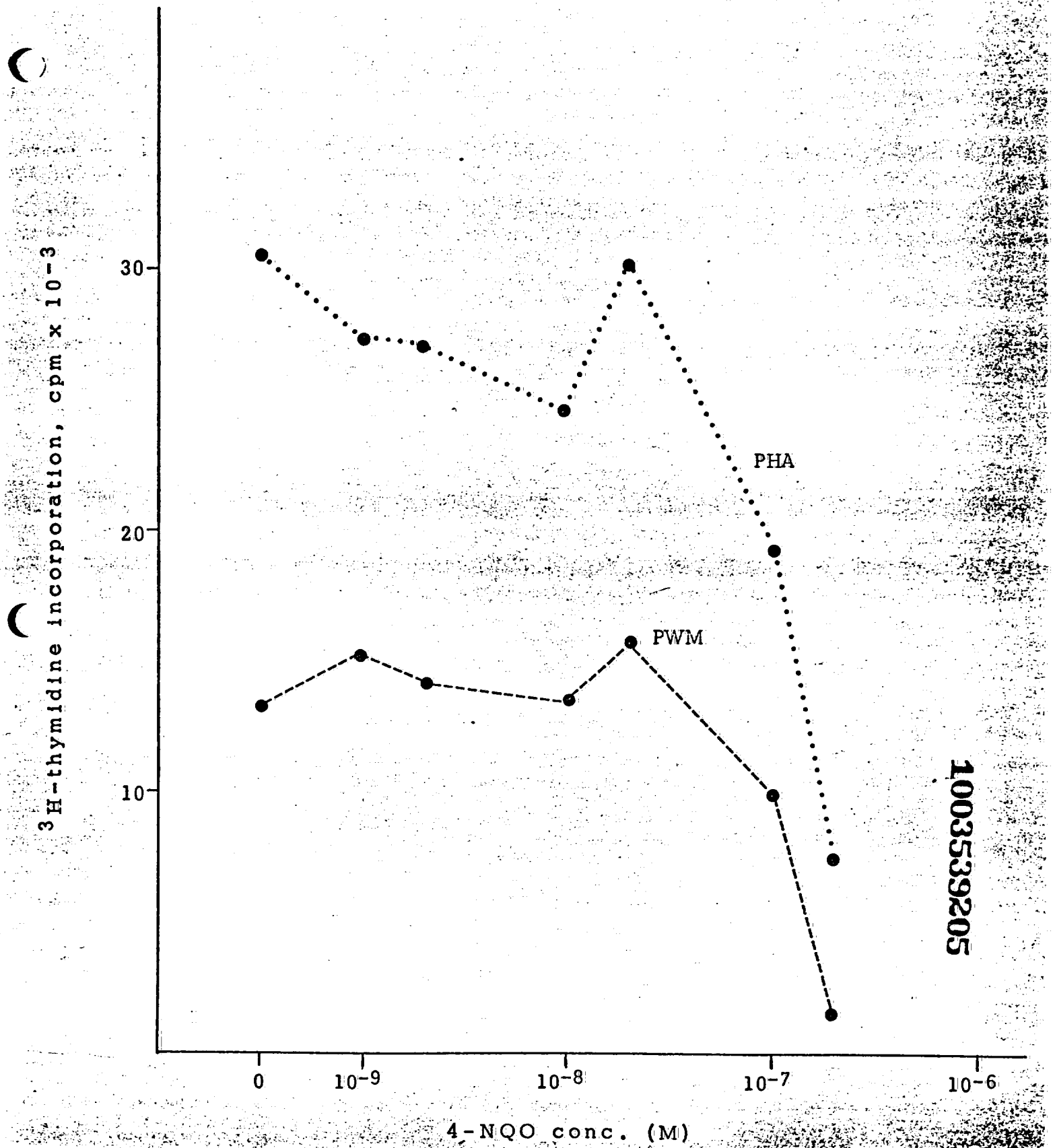

Fig. 4

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Recent Pertinent Publications*:

1. Parker, J.W., O'Brien, R.L., Lukes, R.J. and Steiner, J.
Transformation of human lymphocytes by sodium periodate.
Lancet 1:103-104, 1972.

2. Jacobs, A.J., O'Brien, R.L., Parker, J.W. and Paolilli, P.
DNA repair following exposure of human lymphocytes to 4-nitroquinoline-1-oxide.
Internat. J. Cancer 10:118-127, 1972.

3. Parker, J.W., O'Brien, R.L., Lukes, R.J., Steiner, J. and Paolilli, P.
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Immunol. Comm. 1:263-277, 1972.

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Periodate induced lymphocyte transformation. 2. Character of response and comparison with phytohemagglutinin and pokeweed mitogen stimulation.
Exptl. Cell Res. (Accepted for publication.)

5. Jacobs, A.J., O'Brien, R.L., Parker, J.W. and Paolilli, P.
Abnormal DNA repair of 4-nitroquinoline-1-oxide induced damage by lymphocytes in xeroderma pigmentosum.
Mutat. Res. 16:420-424. 1972.

In preparation:

Parker, J.W., O'Brien R.L. and Steiner, J.
Periodate induced lymphocyte transformation. 3.

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THE COUNCIL FOR TOBACCO RESEARCH – U.S.A., INC.

February 8, 1973

P=5

Grant Application No. 847R1

To: The committee comprising Drs. Huebner, Meier and Sommers

Subject: Wayne L. Ryan, Ph.D., University of Nebraska College of Medicine, Omaha, Nebraska
Renewal application No. 847R1
"The 3', 5' Cyclic Monophosphate System in Carcinogenesis"

History

A three year project was approved in 1972. The current grant is for $53,692.

The present request, for $55,261, is exactly the amount initially estimated. One more year of "priority in competition" remains.

Documents Submitted (attached)

1. Application dated 1-8-73.

2. Progress Report, July 1, 1972 - July 1, 1973.

Comment

The bulk of the Progress Report appears to be the Ryan and Curtis paper at the Fifth Annual Miami Winter Symposia, No. 2 under item 11 on page 2 of the application.

F.W.N.

FWN:wg
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THE COUNCIL FOR TOBACCO RESEARCH—U.S.A., INC.

110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

Application For Renewal of Research Grant

(Use extra pages as needed)

First Renewal [X] Second Renewal [ ] Date: 1-8-73

1. Principal Investigator (give title and degrees):

Wayne L. Ryan, Ph.D. - Professor of Biochemistry

2. Institution & address:

University of Nebraska College of Medicine
42nd and Dewey Avenue
Omaha, Nebraska 68105

3. Department(s) where research will be done or collaboration provided:

Department of Biochemistry

4. Short title of study:

The 3',5' Cyclic Monophosphate System in Carcinogenesis

5. Proposed renewal date: July 1, 1973

6. How results to date have changed earlier specific research aims:

The transport of cyclic AMP is going to be investigated in addition to the previously stated specific aims.

7. How results to date have changed earlier working hypothesis:

The level of cyclic AMP is not lower in tumor cells than in normal cells. If anything, our results indicate that cyclic AMP may be higher in tumor cells. However, for each cell there is a normal level of cyclic AMP, and a decrease from that level is accompanied by an increase in cell division; an increase in the level of cyclic AMP is accompanied by a decrease in cell division.

One alteration in the cyclic AMP system not considered previously is transcellular transport. Our studies indicate that either viral or chemical carcinogenesis results

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7. (continued)

in a cell that does not effectively transport cyclic AMP. This may represent the loss of an important control mechanism because the tumor cell would not receive the same cyclic AMP signals from adjoining cells to cease division, and it would receive inadequate cyclic AMP to maintain a differentiated state. This hypothesis would be consistent with the changes found in membrane structure in the normal and malignant cell.

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Source: https://www.industrydocuments.ucsf.edu/docs/yjbf0000

8. Any additional facilities now required? Describe briefly:

None

9. Any changes in personnel? Append biographical sketches of new key professional personnel:

None

10. Append outline of experimental protocol for ensuing year.

11. List publications or papers in press resulting from this or closely related work. (append reprints or manuscripts not previously sent).

1. Ryan, W. L. & McClurg, J. E., Tissue Levels of Cyclic AMP and Tumor Inhibition, Conf. on Cyclic AMP and Immune Responses, Marco Island, Florida, Jan. 7-10, 1973 (in press).
2. Ryan, W. L. and Curtis, G. L., Chemical Carcinogenesis and Cyclic AMP, The Fifth Annual Miami Winter Symposia, Miami, Florida, Jan. 15-19, 1973, Academic Press (in press).
3. Ryan, W. L., Cancer and Cyclic Nucleotides, for Volume 4 of Advances in Cylic Nucleotide Research, Raven Press, 1973 (in preparation).

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12. Summary progress report (append in standard form as separate document, unless recently submitted).

Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

Outline of Goals for Next Year:

1. Compare the transport of cyclic AMP by each of the cell lines and their percent inhibition by each.
2. Compare the activation of adenylate cyclase in the normal and carcinogen-treated cell lines.
3. Compare the phosphodiesterases of each of the cell lines.
4. Compare the protein kinase-binding protein of each of the cell lines.

The experimental methodology is described in our initial report.

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13. Budget for the coming year:

A. Salaries (give names or state "to be recruited")	% time	Amount
Professional (give % time of investigator(s) even if no salary requested)		
Margaret L. Heidrick, Ph.D. Instructor	100%	13,922
Technical		
TBA, Dishwasher	100%	4,741
TBA, Secretary	25%	1,524
Neal A. Short, Technician	100%	8,466
	Sub-Total for A	$ 28,653

B. Consumable supplies (by major categories)

Protein Kinase Assays $6.00/assay (400 assays)--	$ 2,400
Cyclic AMP $7.50/assay (400 assays)----------	3,000
Phosphodiesterase $2.00/assay (400 assays)------	800
Tissue Culture Supplies----------	11,000
Miscellaneous Supplies----------	1,500
Sub-Total for B	$ 18,700

C. Other expenses (itemize)

Travel	$ 500
Publication Costs	$200
Sub-Total for C	700
Running Total of A + B + C	$ 48,053

D. Permanent equipment (itemize)

Sub-Total for D	
E	7,208
Total request	$55,261

E. Indirect costs (15% of A+B+C)

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

14. Other sources of financial support:

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Glycine in Asparaginase Inhibition of Lymphosarcoma	National Inst. of Health #CA12110-02	$ 26,994	4/1/72-3/31/73
Assay System for Predicting the Effectiveness of Asparaginase	John A. Hartford Foundation	$ 25,818	12/1/72-11/30/73
Regulation of Cell Division by Cyclic AMP	American Cancer Society # BC-48A	$ 30,774	1/1/73-12/31/73

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name Wayne L. Ryan, Ph.D.

Signature Wayne L. Ryan Date 1-9-73

Telephone 402 - 541-4445
Area Code Number Extension

Checks payable to

University of Nebraska Medical Center

Mailing address for check:

Director of Finance, Univ. of Nebr. Med. Center

42nd & Dewey Ave., Omaha, Nebr. 68105

Responsible officer of institution

Typed Name Robert D. Sparks, M.D.

Title Chancellor, Univ. Nebr. Med. Center

Signature [signature] Date 1/14/73

Telephone 402 541-4200
Area Code Number Extension

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyb0000

CTR GRANT # 847

PROGRESS REPORT NO. 1

University of Nebraska
College of Medicine
42nd and Dewey Avenue
Omaha, Nebraska 68105

Dates covered:

July 1, 1972 - July 1, 1973

3',5' Cyclic Monophosphate System in Carcinogenesis

Summary of Findings to Date:

The cyclic AMP system of the following cells is under investigation:

Fischer Rat Embryo - normal fibroblast, F111

Fischer Rat Embryo - 3-MC transformed, F2412

Fischer Rat Embryo - RLV transformed, F1849

Fischer Rat Embryo - High passage, non-transformed, F1706

Lakeview Syrian Hamster - transformed by fraction 9 from cigarette tars

Lakeview Syrian Hamster - normal control

Adenylate cyclase of the cells was measured (basal and fluoride stimulated) throughout the growth cycle. The basal level of the carcinogen transformed cells rises when the cells reach confluency, whereas the normal control cells continue to decrease. Treatment of the cells with trypsin, standard procedure to remove the cells from the petri dish surface, causes a considerable increase in the adenylate cyclase of the normal cell, but little or no change in the carcinogen-treated cells. This effect of trypsin suggests an alteration in the membrane of the carcinogen-treated cell has occurred.

The cyclic AMP pool of the cells was measured throughout the growth cycle. As might be expected from the elevated adenylate

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cyclase, the cyclic AMP level of all the cells is increased at 0 and 8 hours, and then starts decreasing. This decrease continues throughout the growth cycle, and the lowest levels are found at confluency. This is definitely not an expected result in view of previous data obtained by us and others.

Cyclic AMP inhibition curves were established for each of the cell lines. A very marked difference in the response of the normal and carcinogen-treated cells to cyclic AMP was observed. The normal cells are inhibited as much as 90% in cell division by cyclic AMP, whereas the carcinogen-treated cells are unaffected by cyclic AMP. Further investigation suggests this is due to the decreased ability of the tumor cells to transport cyclic AMP. This is particularly interesting because the inability to transport cyclic AMP may be the loss of an important control mechanism. Much of the control of cell division in an organ or tissue may result from the transcellular transport of cyclic AMP. A cell unable to transport cyclic AMP would lose the inhibiting and differentiating effects of cyclic AMP that is common to all the normal cells in the tissue.

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Source: https://www.industrydocuments.ucsf.edu/docs/gyjl0081

CHEMICAL CARCINOGENESIS AND CYCLIC AMP

Wayne L. Ryan and Gary L. Curtis
University of Nebraska College of Medicine
Omaha, Nebraska 68105

The apparent rigorous control which is exercised over cell division in each organ led to the assumption that there exist substances in tissues which regulate cell division. This hypothesis usually has suggested that such compounds should act as inhibitors and that cells will divide at a maximal rate in the absence of the inhibitor. A search for such substances led us to investigate adenosine 3',5'-monophosphate (cyclic AMP) as a potential regulator of cell division.

Cyclic AMP was found to inhibit cell division in tissue culture and to do so in a unique manner. There is no apparent toxicity, such as detachment from the glass, even at concentrations of up to 3 mM. Removal of the cyclic AMP-containing medium permits the cells to divide again in a normal manner (1). The variety of means available for elevating the levels of cyclic AMP by means of adenylate cyclase and lowering the levels by phosphodiesterase permit cyclic AMP the requited flexibility to carry out this possible role as a regulator of cell division.

Considerable evidence has accumulated which suggests a correlation between the cellular level of cyclic AMP and the rate of cell division (2-5). A further development has been the comparison of the levels of cyclic AMP, or one or more of the enzymes of the cyclic AMP system in normal, transformed, or malignant cells (6-14). At present, most evidence indicates that the rapidly dividing cell has lower levels of cyclic AMP, and for this reason it is concluded that the transformed and malignant cell may have defects in the cyclic AMP system.

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Source: https://www.industrydocuments.ucsf.edu/docs/xylf0000

Although transformation of cells in vitro results in a number of interesting cellular changes, none of these changes seem to be uniquely associated with tumorigenicity or neoplasia (15, 16). For this reason, cell lines of known tumorigenicity and lineage were sought. An appropriate cell system for studying the changes in the cyclic AMP system following chemical carcinogenesis is that of Freeman et al (17). A normal rat embryo cell line, F111, free of murine viruses, was transformed in vitro by a variety of chemical carcinogens and by the Rauscher Leukemia Virus. The resultant cell lines produce tumors in the neonatal rat. In addition, the normal rat embryo cell of origin is available for comparison with the tumorigenic cell lines (Figure 1).

The aim of this study is to assay the cyclic AMP system in normal and carcinogenic treated cells. In addition, investigations of the response of the tumor cell in vitro to cyclic AMP and cyclic AMP elevating agents may provide a rational basis for an approach to inhibiting the growth of the tumors produced in vivo by these cell lines.

MATERIALS AND METHODS

Cell Culture

The rat embryo cell lines (F111 p. 32-34, F1706 p. 118-122, F1849 p. 59-62, and F2412 p. 36-37) were seeded in 20 X 100 mm plastic culture dishes (Falcon) at a cell density of 5000/cm^2 in Dulbecco's Modified Eagle's Medium with glutamine (Grand Island Biological) with 10% fetal calf serum. To the medium was also added sodium bicarbonate (13.5 g/1), amphotercin B (2 mg/1), penicillin (50,000 units/1), and streptomycin (500 mg/1). The cells were incubated at 37° in an atmosphere of 5% CO_2 and 95% air. The cells were removed from the dishes with 0.25% trypsin. All cells were counted on a Model Fn Coulter Counter.

The inhibition studies were conducted in 60 mm petri dishes containing 5 ml. of Dulbecco's Modified Eagle's Medium with 10% fetal calf serum. The percent inhibition=

$$\frac{\text{Cell count (control)} - \text{cell count (treated)}}{\text{Cell count (control)}} \times 100$$

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Source: https://www.industrydocuments.ucsf.edu/tobacco/

Adenylate Cyclase

After removing the culture medium, the cells were washed with 10 ml. of 0.85% saline. For values obtained at 24 hours, 48 hours, and 96 hours of incubation, the cells were removed by scraping. For the zero time value the cells were removed by trypsinization and 3 ml. of fetal calf serum added to inhibit the action of trypsin. After removal, the cells were centrifuged and the cells were prepared as described by Makman with the exception that 5 mM dithioerythritol was added to the buffer (13, 17).

The incubation conditions were modified from those described by Makman and Krishna and were determined to give linear rates of activity for 20 min. at 30° C (13, 17, 18). Incubations were carried out in 10 X 75 mm test tubes containing: 1.0 mM ^3H ATP, 4.5 mM $MgCl_2$, 2.0 mM cyclic AMP, 2.5 mM phosphoenolpyruvate, 3.0 mM theophylline, 1.25 mg/ml pyruvate kinase, 45 mM Tris-HCl pH 7.6, and either 8 mM NaF or the Tris-Mg buffers in a final volume of 200 µl. The reactions were started by addition of cell homogenates to give final concentrations ranging from 1.0 to 2.0 mg. protein per milliliter of assay. Incubations were carried out at 30° C and were terminated by the addition of 0.2 milliliter of 50 mM Tris/HCl, pH 7.6, 5 mM cyclic AMP, and 3 mM ATP. The reaction mixtures were heated for two minutes in a boiling water bath after which 0.6 ml. of distilled water was added per tube. The cyclic AMP was isolated and measured as described by Krishna (18).

Cyclic AMP Assay

The medium was removed from the cells and drained as completely as possible. To each dish of cells was added 5 ml. of 0.5 N perchloric acid in 25% ethanol (v/v) containing tracer cyclic AMP for estimation of recovery. The precipitated cell sheet was scraped, washed, and centrifuged. The supernatant was neutralized with saturated KOH. After removal of the precipitate, the supernatant was chromatographed by the method of Brooker (19). The cyclic AMP assay was used as described by Gilman (20). A sample from each series was treated with phosphodiesterase

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as described by Otten et al (4).

Cell Volume Determinations

For cell volume determinations, the growth media was removed from exponentially growing monolayer cultures and replaced with fresh growth media containing the test reagents. Following incubation, the cells were washed with 2 ml. Dulbecco's Tris buffer (0.025 M Tris, 0.1 M KH_2PO_4, and 0.25 M saline, pH 6.8-7.0) then incubated with 1 ml. of 0.25% trypsin for 5 minutes at 37°. The detached cells from each plate were placed in 100 ml. of Dulbecco's Modified Eagle's Medium, pH 7.2, (containing 0.04 M Tris buffer) supplemented with 10% fetal calf serum, penicillin (500 units/ml.), and streptomycin (500 g/ml.) and the cell volume measured with a Model B Coulter Counter.

RESULTS

In Figure 1 is a summary of some of the characteristics of four of the rat embryo cell lines. The most rapidly dividing cell is the F2412, which was transformed by 3-methylcholanthrene. The cell volume of the two transformed cell lines (F2412 and F1849) is very similar, and the largest of the four is the normal cell line, F111. The F2412 and F1849 produce tumors in the neonatal rat, whereas the F111 and F1706 do not.

The basal and fluoride stimulated levels of adenylate cyclase of each of the four cell lines is compared in Table 1. The only remarkable difference in the values is the elevation of the basal level of the F2412 at 120 hrs. The elevation of the basal values at zero time must be due to the effect of trypsinization or the addition of fresh serum, which is added to inhibit the trypsin, since the zero time values are obtained by trypsinization of 120-hour cultures. Since the fluoride affects ATPase and adenylate cyclase, which are membrane bound enzymes, presumably the chemical carcinogen has altered the membrane of the F2412 cells. Similar elevations are seen in the zero time values for the fluoride stimulated levels of F111, but not F2412. The lowest values for basal and fluoride stimulated levels of adenylate cyclase are found at 18 hours and 48 hours during which time the

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cells are multiplying most rapidly.

Similarly, the highest values obtained for cyclic AMP (Table 2) in the cell lines were found between 0 and 8 hours after trypsinization of the cells. In general, all of the cyclic AMP values decrease after 8 hours reaching their lowest levels at 168 hours. The F2412 and F1849 cells have the lowest levels of cyclic AMP and this is particularly apparent during logarithmic growth. From comparison of the adenylate cyclase and cyclic AMP levels, it appears that a correlation exists between basal levels of adenylate cyclase and cyclic AMP levels except for the 120-hour value of F2412.

The effects of adding exogenous cyclic AMP or dibutyryl cyclic AMP is generally found to retard the rate of division of cells. Because the cells metabolize the added cyclic AMP to 5' AMP, which may also be inhibitory, inhibition is a consequence of both cyclic AMP and 5' AMP. For this reason, it is best to assay the effect of cyclic AMP on cells at several cell densities. This relation between cell density and cyclic AMP inhibition has been demonstrated by van Wijk, Wicks, and Clay (21). The effects of exogenous cyclic AMP on each of the cell lines is shown in Table 3. The tumor cell line F1849 was not inhibited by cyclic AMP and F2412 was only slightly inhibited, whereas the normal cell lines were markedly inhibited. An explanation for this difference was presented by a previous investigation with WI-38 cells which were also found to be resistant to inhibition by cyclic AMP. This resistance is related to a decreased ability to transport cyclic AMP (22, 23). To test this possibility, each of the four cell lines were incubated in 0.3 mM cyclic AMP for 24 hours and the percent inhibition and cyclic AMP level in each of the cells measured. The results in Table 4 show that there is a correlation between the level of cyclic AMP and the inhibition observed. The tumor cells have considerably lower levels of cyclic AMP than the normals, which suggests a possible mechanism for their resistance to exogenous cyclic AMP. This may be due to a decreased transport of cyclic AMP, or may also result from more rapid destruction by phosphodiesterase.

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An attempt to inhibit these tumors in vivo by injection of cyclic AMP would appear futile. However, other agents such as theophylline, the prostaglandins, and dibutyryl cyclic AMP may inhibit the cells. An investigation of the inhibitory effects of these agents is shown in Table 5. Although cyclic AMP does not inhibit the carcinogen or virus transformed cells, theophylline is more inhibitory to these two tumor cells than it is to either of the normal cells. Although dibutyryl cyclic AMP inhibits all four of the cell lines, the addition of equimolar butyrate to the cells is equally inhibitory which probably indicates that the inhibition by dibutyryl cyclic AMP is actually due to the butyrate. The prostaglandin E_1 is more inhibitory to the normal cells than to the tumor cells. The results suggest that theophylline might be more effective than PGE_1 or cyclic AMP in inhibiting the growth of the tumor cells in vivo.

DISCUSSION

The basal adenylate cyclase levels of the 3-methylcholanthrene transformed cell line (F2412) and its cell of origin (F111) are similar except when the cells reach confluency. At confluency the basal level of adenylate cyclase in the F2412 cell line is approximately three times as great as the normal cell. The significance of this increase is difficult to interpret, as the F111 cell line shows a sharp decrease in growth at confluency whereas the F2412 continues to grow and is not density inhibited. To obtain zero time values, cells from confluent cultures were trypsinized. Since this is the only difference in treatment between the 120-hour values and the zero time values for adenylate cyclase, the marked increase in the basal values for this time period must be due to the trypsinization procedure. Although the normal cells show a 7-fold (basal) and 2-fold (fluoride) increase following the trypsinization, the tumor cell (3-MC) is increased only 2-fold in the basal level and in the fluoride level not at all. Effects of trypsin on cell division, cyclic AMP levels, and on membrane phenomenon have been reported. The elevation of adenylate cyclase by trypsinization of normal cells, with slight effect on tumor cells, may also be related to the above phenomena.

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Some correlation is seen between the levels of adenylate cyclase and cyclic AMP in the cell; for example, the highest cyclic AMP and adenylate cyclase values are found after trypsinization. The cyclic AMP levels in the cells decrease from this point and reach their lowest levels at confluency. The lack of correlation between cyclic AMP and adenylate cyclase values, especially in the F2412 cell line, after the first 48 hours may be due to an increase in phosphodiesterase which has been shown to be an inducible enzyme (24, 25). A rise in phosphodiesterase may lower the cyclic AMP levels in spite of the increase in adenylate cyclase. Although the values for cyclic AMP in the four rat embryo cell lines are similar to those reported by others (3), they are at least 10-fold higher than those that we have found for the 6C3HED, L5178Y, and L1210 tumor cells in vivo and nearly 100 times greater than normal mouse liver and spleen (26). The explanation for these considerable differences is not presently clear. Neither of the tumor cells are inhibited by exogenous cyclic AMP, whereas the normal cells are greatly inhibited. A possible explanation for this difference is the observation of lower cellular levels of cyclic AMP found in the tumor cells after incubation in cyclic AMP. This may be due to a reduced ability of cyclic AMP to be transported by the tumor cells or to higher phosphodiesterase levels in the tumor cells. The inability to effectively transport cyclic AMP may represent the loss of an important control mechanism. Cells at high density, as in tissues, may regulate the rate of cell division of each other by inter and intracellular transport of cyclic AMP. Loss of this relationship may permit the tumor cell to grow independently of other cells in the organ without the restraint of cyclic AMP.

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CYCLIC NUCLEOTIDES IN CANCER

SUMMARY

A normal rat embryo cell line was transformed in vitro by 3-methylcholanthrene to a cell which produces tumors in the Fischer rat. The adenylate cyclase, cyclic AMP level during growth in tissue culture and response to exogenous cyclic AMP were investigated. For comparison a high passage rat embryo cell line and a cell line transformed by RLV, arising from the same original cell line were also examined. The carcinogenic transformation results in a cell of smaller cell volume which divides more rapidly. Treatment with trypsin increases the basal level of adenylate cyclase in the normal cell considerably, but alters the adenylate cyclase of the malignant cell only slightly.

The inability of cyclic AMP or dibutyryl cyclic AMP to inhibit the tumor cells is believed to be due to a decreased ability to transport cyclic AMP. This may represent the loss of an important control mechanism in which cells in organized structures control the rate of division of each other.

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Source: https://www.industrydocuments.ucsf.edu/docs/vyb8000

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Table 1

Adenylate Cyclase of Rat Embryo Cell Lines

Basal Level

Time (hours)	F111	F1706	F1849	F2412
0	47.7	13.2	10.2	45.6
18	2.6	3.9	1.3	3.2
48	7.5	7.8	2.3	5.9
120	7.7	8.7	3.4	23.6

Fluoride-Stimulated Level

Time (hours)	F111	F1706	F1849	F2412
0	124.0	43.0	102.0	62.0
18	5.4	11.8	10.3	5.9
48	48.0	29.0	17.0	11.0
120	67.0	59.0	20.0	68.0

Values pmoles cyclic AMP formed/minute/mg. of protein at 30°. Linear rates were obtained for 20 minutes. The 15 minute value was used for the calculations. Values for 0 time obtained by trypsinization of confluent cultures. Logarithmic growth obtained at 18 and 48 hours. Cells were confluent at 120 hours. Cells were plated at a density of $50/mm^2$.

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Source: https://www.industrydocuments.ucsf.edu/docs/gyjd0081

Table 2

Cyclic AMP Level of Rat Embryo Cell Lines During Growth

Hours	F111	F1706	F1849	F2412
0	60	200	336	97
8	102	120	67	43
24	46	84	29	19
48	50	68	18	15
120	34	9	16	16
168	15	3	10	4

Cells were seeded at $50/mm^2$. Values are pmoles/10^6 cells. Average of five determinations. Logarithmic growth occurs at 24 and 48 hours and cells are confluent at 120 hours. To convert pmoles/10^6 cells to pmoles/10^{-1} mg. multiply values by: F111 = 0.42, F1706 = 0.39, F1849 = 0.48, and F2412 = 0.76.

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Source: https://www.industrydocuments.ucsf.edu/docs/kdyl0000

Table 3

Cyclic AMP Inhibition of Rat Embryo Cell Lines

	F2412			F1849	
mM	A	B	mM	A	B
0.3	0	10	0.3	0	0
0.7	18	26	0.7	0	0
1.0	14	28	1.0	0	0

	F1706			F111	
mM	A	B	mM	A	B
0.3	23	48	0.3	14	35
0.7	46	65	0.7	59	65
1.0	57	74	1.0	60	69

Cell density for A = 54 cells/mm^2; B = 27 cells/mm^2.
Percent inhibition 72 hours after plating of cells.

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Source: https://www.industrydocuments.ucsf.edu/docs/xyf000

Table 4

Level of Cyclic AMP in Rat Embryo Cells
After Incubation in Exogenous Cyclic AMP

	Cyclic AMP/10^6 Cells	% Inhibition
F1706	7546	32
F111	5038	20
F1849	3807	0
F2412	2100	0

Cells were plated at a density of 50 cells/mm^2. 0.3 mM cyclic AMP added to each experimental group at the time of plating. Cells were washed six times with cold saline before the addition of perchloric acid. Cyclic AMP levels and percent inhibition determined at 24 hours. Values are an average of four determinations.

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Table 5

Response of Rat Embryo Cell Lines to Theophylline, Dibutyryl Cyclic AMP, Cyclic AMP, and PGE_1

Inhibitor	F2412	F1849	F1706	F111
Theophylline, 1 mM	84	75	26	40
Dibutyryl Cyclic AMP, 1 mM	39	53	54	65
Butyrate, 1 mM	39	38	48	68
Cyclic AMP, 1 mM	10	0	65	62
PGE_1 25 µg/ml.	13	0	20	44

Percent inhibition determined after 72 hours incubation. Cells were plated at a density of 50 cells/mm^2.

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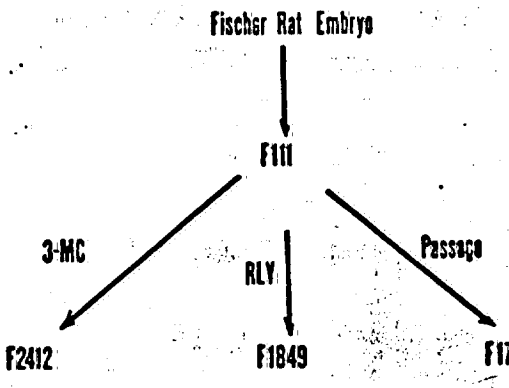
Source: https://www.industrydocuments.ucsf.edu/docs/jtyl0000

FIGURE 1. ORIGIN AND CHARACTERISTICS OF RAT EMBRYO CELL LINES



	POPULATION DOUBLING TIME	PASSAGE NUMBER	CELL VOLUME μ^3	TUMORIGENICITY NEONATAL RAT
F111	23	32-34	3200	No
F1706	20	118-122	2100	No
F2412	15	36-37	2600	Yes
F1849	23	59-62	2500	Yes

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

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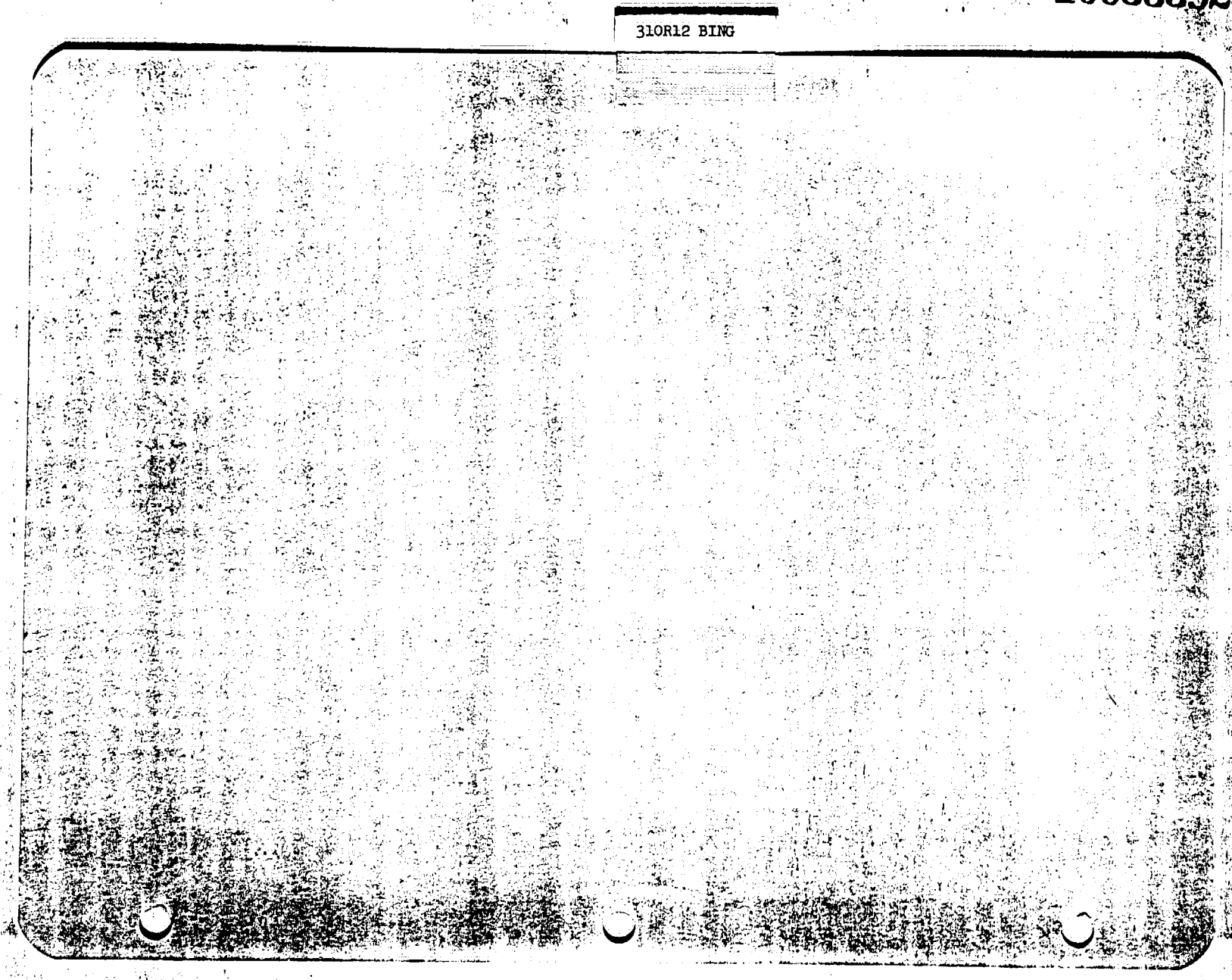

Source: https://www.industrydocuments.ucsf.edu/docs/vtyl0000

THE COUNCIL FOR TOBACCO RESEARCH – U.S.A., INC.

February 5, 1973

P-1

Grant Application No. 310R12

To: The committee comprising Drs. Cattell, Jacobson and Sommers

Subject: Richard J. Bing, M.D., Huntington Memorial Hospital, Pasadena, California
Renewal application No. 310R12
"Carbon Monoxide and Coronary Atherosclerosis"

History

CTR support of Dr. Bing goes back to 1955. The grant now in effect is for $29,325.

This request is for $38,893.

Documents Submitted (attached)

1. Application dated 1/31/73, nine pages plus C.V. of James C. Mao, Ph.D.

2. Progress report June 30, 1972 - July 1, 1973, seven pages plus bibliography.

FWN

F.W.N.

FWN:wg
Encls.

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Source: https://www.industrydocuments.ucsf.edu/docs/ydyl0000

Comm.
Dr. Cattell
Dr. Jacobson
Dr. Sommers

CARDIOVASCULAR

#310R12
(Renewed annually since 7/1/62)
#CF #91 - 7/1/55
#CF #270-7/1/60-7/1/61

THE COUNCIL FOR TOBACCO RESEARCH–U.S.A., INC.
110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

FEB 1 1972

Application For Renewal of Research Grant
(Use extra pages as needed)

First Renewal ☐ Second Renewal ☐ Date: 1/31/73

1. Principal Investigator (give title and degrees): Richard J. Bing, M.D.
Professor of Medicine, University of Southern California
Director of Cardiology and Intramural Medicine,
Huntington Memorial Hospital
Visiting Associate in Biomedical Engineering,
California Institute of Technology

2. Institution & address:

Huntington Memorial Hospital
100 Congress Street
Pasadena, California 91105

3. Department(s) where research will be done or collaboration provided:

Huntington Institute of Applied Medical Research
Huntington Memorial Hospital

4. Short title of study:

Carbon Monoxide and Coronary Atherosclerosis

5. Proposed renewal date: July 1, 1973

6. How results to date have changed earlier specific research aims:

The research aims have expanded to include carbon monoxide. No change in the general specific aims have resulted from our findings.

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7. How results to date have changed earlier working hypothesis:

Our working hypothesis has been to study human coronary atherosclerosis by using human coronary arteries. Our results are included in our progress report.

Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

8. Any additional facilities now required? Describe briefly:

None

9. Any changes in personnel? Append biographical sketches of new key professional personnel:

The biochemical execution of this work is under the supervision of Dr. James C. Mao, whose curriculum vitae is enclosed. The overall direction of this project remains with Dr. Richard Bing, the principal investigator.

10. Append outline of experimental protocol for ensuing year. See enclosed.

11. List publications or papers in press resulting from this or closely related work. (append reprints or manuscripts not previously sent).

See enclosed.

12. Summary progress report (append in standard form as separate document, unless recently submitted).

See enclosed.

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

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13. Budget for the coming years

A. Salaries (give names or state "to be recruited")	% time	Amount
Professional (give % time of investigator(s) even if no salary requested)		
James C. Mao, Ph.D.	90	
Fringe Benefits		REDACTED
H. Tillmanns, M.D.	30	
J. M. Fauvel, M.D.	30	
R. J. Bing, M.D.	70	
Technical		
E. Larsen, B.S.	100	
Fringe Benefits		REDACTED
K. Seeler, M.S.	70	
H. Hansen, B.A.	30	
Sub-Total for A		
B. Consumable supplies (by major categories)		
Isotopes, reagents, enzymes, catheters, syringes, general chemicals		9,000
Sub-Total for B		9,000
C. Other expenses (itemize)		
Expenses in obtaining arteries and veins and for histological examinations		1,000
Sub-Total for C		1,000
Running Total of A + B + C		$33,298
D. Permanent equipment (itemize)		
Lindbergh pump		600
Sub-Total for D		600
E. Indirect costs (15% of A+B+C)		4,995
Total request		$38,893

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

14. Other sources of financial support.

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
"The Direct Effect of Nicotine and Catecholamines and Their Agonists and Antagonists on the Coronary Microcirculation"	American Medical Association Education and Research Foundation	$33,312	11/1/72 to 10/30/73
"The Effect of Alcohol on the Heart"	National Institutes of Health, MH21052-02	$38,700	1/1/73 to 12/31/73

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name Richard J. Bing, M.D.

Signature Richard J. Bing M.D. Date 1/30/73

Telephone (213) (Area Code) 796-0371 (Number) 526 (Extension)

Checks payable to

Huntington Memorial Hospital

Mailing address for check:

Huntington Memorial Hospital

Pasadena, California 91105

Responsible officer of institution

Typed Name Mel Ortmann

Title Business Manager

Signature Mel Ortmann Date 1/30/73

Telephone (213) (Area Code) 796-0371 (Number) 510 (Extension)

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

APPLICATION FOR RENEWAL OF RESEARCH GRANT

Richard J. Bing, M.D.
Huntington Memorial Hospital
100 Congress Street
Pasadena, California 91105

Project Title: The Effect of Carbon Monoxide on Atherosclerosis of Coronary Arteries and Saphenous Veins of Man (In Vitro Studies)

Background of Proposed Work

As described in our progress report, we have studied the lipid metabolism in perfused human atherosclerotic and nonatherosclerotic coronary arteries and saphenous veins. In atherosclerotic coronary arteries, it could be established that these vessels cannot synthesize cholesterol from acetate, that only very limited synthesis of cholesterol esters from acetate occurs, that free cholesterol is taken up by the artery, and, finally, that nicotine has a very slight effect on lipid synthesis from acetate but does not influence free cholesterol uptake.

Our experiments then were extended to include nonatherosclerotic human coronary arteries and saphenous veins. The arteries were obtained within five hours following death of the patient and the saphenous veins at the time of operation for aortocoronary bypass.

The results obtained on nonatherosclerotic human coronary arteries were quite similar to those obtained on the diseased vessels. Consequently, human coronary arteries without evidence of atherosclerosis did not differ from the atherosclerotic vessels in their inability to synthesize cholesterol from acetates;

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

coronary arteries with and without atherosclerotic lesions take up cholesterol from the perfusion fluid to the same degree; human saphenous veins perfused at relatively low pressure (45/35 mmHg) did not differ from atherosclerotic and normal coronary arteries in their ability to synthesize lipids; the uptake of cholesterol was less than that of coronary arteries or saphenous veins perfused at arterial pressure of 130/100 mmHg; human saphenous veins perfused at arterial pressure take up cholesterol from the perfusion fluid equal to that taken up by the coronary arteries; and, in saphenous veins perfused at high pressure, synthesis of lipids from acetate equals that in saphenous veins perfused at lower pressure of nonatherosclerotic coronary arteries. In these experiments, we could demonstrate that nicotine failed to influence either synthesis of lipids or uptake of cholesterol in veins perfused at 45/35 mmHg.

From these two studies we concluded that nicotine has no appreciable influence on either synthesis of lipids in human coronary arteries or veins or on the uptake of cholesterol and cholesterol esters by these vessels.

It was of further interest that there is no synthesis of cholesterol in either coronary arteries or veins, but that cholesterol uptake in arteries and veins is considerable, particularly if the veins are perfused at arterial pressures. This latter point may be of importance in assessing the future of aortocoronary bypass, which is now commonly practiced on patients with coronary artery disease.

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

Proposed Work

The aim of our proposed investigation is a study of the effect of carbon monoxide on lipid metabolism in perfused coronary arteries and saphenous veins.

The effect of carbon monoxide on the production of atherosclerosis has been discussed by a number of investigators. It has been questionable, however, whether this effect is due to hypoxia or to a specific effect of carbon monoxide. Astrup (Journal of Atherosclerosis Research, 7:343, 1967) exposed an experimental series of rabbits to small concentrations of carbon monoxide and a control series to room air. He observed (1) that the degree of visible aortic atheromatosis and the content of total cholesterol in the aortic tissue were significantly higher in the rabbits exposed to carbon monoxide than in the control, and (2) that, in about three fourths of the carbon-monoxide-exposed rabbits, the hearts also displayed pathologic changes. Microscopic examination also supported these findings; it was found that the underlying biochemical and physiological mechanisms may be explained by tissue hypoxia due to a carbon-monoxide-induced displacement of the oxygen dissociation curve to the left, in combination with a decreased activity of certain enzymes inhibited by carbon monoxide.

In another study, the same group of investigators has determined the cholesterol level of aortic tissue in the control group and in animals exposed to carbon monoxide and found that

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

the amount of this lipid was about five times as high as that in the control group. In line with this observation were macroscopic findings which demonstrated that the vascular wall showed various amounts of lipid depcsits. This lipid material was predominantly seen in the intima, which appeared markedly thickened. The coronary vessels of all dimensions showed similar changes. Astrup and coworkers believe that these alterations are due to a more general change of the vessel wall. They are of the opinion that CO increases capillary permeability, as first postulated by Kjeldsen and Giese. This hypothesis, that the alterations in the vascular wall induced by carbon monoxide are due to an increase in vascular permeability, was confirmed by Andersen and his coworkers (Andersen, et al., Scandinavian Journal for Clinical and Laboratory Investigation, 22:39, 1968). They tested the permeability of these vessels by means of penetration of labelled albumin (^{131}I-albumin). Whether the studies of Astrup and associates are valid is as yet doubtful. Astrup and coworkers (Atherosclerosis) found changes similar to those which are produced by carbon monoxide in animals exposed to hypoxia.

In this country, most of the studies on the effect of carbon monoxide on cardiovascular disease are based on the work of Aronow. This investigator found in 1971 (Circulation, 54:782, 1971) that, following the smoking of non-nicotine cigarettes, the mean carboxyhemoglobin level rose from 1.6 to 7.8%.

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

Smoking significantly decreased the mean exercise time from the onset of exercise until the onset of angina by a considerable range. One particular finding is of interest, namely that smoking non-nicotime cigarettes increased the carboxyhemoglobin level, decreasing the rate of oxygen-carrying capacity to the myocardium; as a result angina develops sooner with less cardiac work. This particular statement is surprising because, in another study published in the Annals of Internal Medicine, 74:697, 1971, the same author analyzes the effects of smoking on the cardiovascular system. He concluded that the main hemodynamic alterations are due to the effect of nicotine and not to that of carbon monoxide. Finally, in a recent paper (Annals of Internal Medicine, 77:669, 1972) Aronow and coworkers found that freeway traveling markedly increased carbon monoxide concentration in the blood and diminished exercise performance until angina appeared. He also observed ischemic ST-segment depression in three of his ten patients while breathing freeway air. Roughly, the arterial carboxyhemoglobin level increased from about .8 to about 7.5%.

The main conclusions drawn from these studies in the literature are as follows: (1) The effect of carbon monoxide on atherosclerosis may be a nonspecific effect similar to that induced by hypoxia. This has been demonstrated by increased permeability under both sets of experimental conditions. (2) The hemodynamic effects of smoking are in all likelihood not the result of carbon monoxide but of nicotine.

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

These changes also suggest that carbon monoxide has an effect on permeability of the vascular wall. Chance has shown that there are two different carbon monoxide targets, both of which involve changes in hematin-iron linkage. The first target exists at the blood level, where the reaction of carbon monoxide with hemoglobin creates carboxyhemoglobin, which is inactive in oxygen transport; the second exists at the cellular level where the hematin-iron atoms of ^{3}A-cytochrome are liganded by CO, thereby losing their reactivity toward oxygen. The former target has been the subject of extensive investigations by many physiologists; the latter remains the domain of the biochemist. It is likely that carbon monoxide makes itself felt in both directions, (a) through the effect of the dissociation curve, and (b) through the mitochondrial-electron transfer chain. Chance explains the latter as a loss of the ability of the matrix of enzymes involved in membrane-bound electron flow to be activated effectively to establish redox patterns of optimal effectiveness by remaining uninhibited cytochrome molecules, thus enabling the flow of oxidizing equivalents to spread through the totality of the membrane components. He emphasizes the extreme sensitivity of mitochondria in the uncoupled or metabolically active state to small concentration of carbon monoxide.

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Method of Procedures

Human coronary arteries (atherosclerotic and nonatherosclerotic) and saphenous veins will be perfused as described in our previous

Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

applications (see progress report). In brief, these arteries will be perfused in a modified Carrel-Lindbergh pump at pulsatile pressure. Arteries will be perfused at pressures of 120/80 mmHg, and veins will be perfused at a pressure below 35 mmHg. In contrast to previous work, where the perfusion fluid consisted of human plasma, we plan to use diluted fresh human blood. The reason for this change in technique is that alterations in oxygen dissociation curve would of course not occur in the absence of the respiratory pigment. On the other hand, in later experiments, it may be possible to perfuse the vessels with plasma alone in order to test the hypothesis of Chance on the effect of carbon monoxide on the mitochondrial respiratory chain alone.

As previously stated, sterile techniques will be used during the preparation and perfusion, and perfusion will be carried out over a period of 4 hours at 37°C. The gas which will drive the fluid through the artery and which will come into equilibrium with the perfusion fluid will consist of 5% CO, 5% CO_2, 20% O_2, and 70% N_2O. In the control experiments, CO will be omitted. It is assumed that this will give an approximate concentration in the perfusion fluid of carbon monoxide of about 15%. To the perfusion fluid will be added 2-^{14}C-sodium acetate and cholesterol-1,2-^{3}H. Cholesterol will be brought into solution by sonication, as described in previous reports. It has been shown that the tritium radioactivity is

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

located primarily in the alpha-2-lipoprotein and in the beta-lipoprotein fraction. Lipids will be analyzed in the perfusion fluid prior to and following perfusion. Analysis will be carried out on the whole blood vessel, and the extraction of the lipids will be carried out according to the method of Folch. Separation of the lipids will be accomplished by means of thin-layer chromatography on silica gel according to the method of Freeman and West. Radioactivity of the eluate will be determined in a scintillation vial and counted in a tricarb liquid scintillation spectrometer. The method of Zak will be used for the determination of cholesterol in plasma extract in the eluate. Phospholipids are analyzed according to the method of Lowry modified by Wagner. The blood vessels will then be analyzed for lipid synthesis as well as for cholesterol and cholesterol ester uptake, and statistical analysis will be used to see whether any significant differences exist between blood vessels perfused under normal conditions and those with perfusion fluid containing carbon monoxide hemoglobin.

Significance of This Work

The significance of this work is twofold. First, it will permit further investigations on the mechanisms of atherosclerosis in human vessels. Since most of the studies concerning the mechanism of coronary atherosclerosis have been performed on animals, the use of human blood vessels constitutes a certain advantage.

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

Second, the question of the effect of carbon monoxide on the mechanism of atherosclerosis has so far been unsettled. Studies have either been carried out on animals, or they have only been restricted to the synthesis of cholesterol in animal blood vessels. Therefore, although the main thrust of this project is directed toward the effect of carbon monoxide, it has more general implications for the overall field of coronary atherosclerosis.

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

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CURRICULUM VITAE

James C. Mao, Ph.D.

PERSONAL
REDACTED
REDACTED

Birthplace:

REDACTED

EDUCATION

B.S., National Taiwan University

M.S., North Dakota State University

Ph.D., North Dakota State University

REDACTED

Postdoctoral fellow (1971-1972) under Dr. Rubin Bressler, Department of Pharmacology, College of Medicine, University of Arizona, Tucson, Arizona

PUBLICATIONS

1. Huxtable, R., Mao, J., Hayes, J. S., Bressler, R., and Picchioni, A. L. 1972. Biochemical changes in the sarcoplasmic reticulum of muscle from andiogenically epileptic rats upon seizure. (Submitted to the Journal of Clinical Investigation).

2. Hashimoto, H., Sarma, J. S. M., Mao, J., Larsen, E., Tillmanns, H., and Bing, R. J. 1973. Lipid metabolism in perfused human nonatherosclerotic coronary arteries and saphenous veins. (Submitted to Atherosclerosis).

3. Pachinger, O. M., Mao, J., Hashimoto, H., Hellberg, K. D., Tillmanns, H., and Bing, R. J. 1973. The effect of prolonged administration of ethanol on cardiac metabolism and performance in the dog. (Submitted to the Journal of Clinical Investigation).

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

CTR GRANT # 310

PROGRESS REPORT NO. 3

RICHARD J. BING, M.D.
Huntington Memorial Hospital
100 Congress Street
Pasadena, California 91105

June 30, 1972 - July 1, 1973

THE EFFECT OF NICOTINE ON LIPID SYNTHESIS AND CHOLESTEROL TRANSFER IN HUMAN CORONARY ARTERIES AND HUMAN SAPHENOUS VEINS

The primary purpose of our work, as sponsored by the Council for Tobacco Research, was to investigate the effect of nicotine on lipid metabolism in human coronary arteries. Special emphasis of this work was on human blood vessels, since considerable differences exist in lipid metabolism of arteries in various species. The emphasis on human material introduced some difficulties; although it was possible to obtain sufficient atherosclerotic human coronary arteries, the supply of normal nonatherosclerotic human coronary arteries was limited. However, it was finally possible to obtain sufficient data to compare diseased and normal human coronary arteries.

We also extended our investigation to the effect of nicotine on the lipid metabolism in perfused coronary saphenous veins. The reason for this was twofold: (1) saphenous veins are more readily available, and we found that the lipid synthesis in the veins differs little from that of coronary arteries; (2) saphenous veins are now frequently employed in the construction of aortocoronary vein grafts,

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Source: https://www.industrydocuments.ucsf.edu/docs/yfh0000

in order to furnish an increased blood supply to the ischemic heart muscle of the patient with coronary artery disease. Several publications have resulted from these studies, one published in the Proceedings of the Society of Experimental Biology and Medicine, the other (enclosed) submitted to Atherosclerosis.

The first portion of this project was concerned with a study of lipid synthesis and cholesterol transfer into atherosclerotic human coronary arteries. The technique used in these studies consisted of sterile perfusion in a modified Carrel-Lindbergh perfusion apparatus of arteries obtained within 5 hours after death of the patient. Perfusion was carried out for 4 hours with a pressure of 130/100 mmHg and a pulsatile rate of 80. The advantage of the Lindbergh pump is that it is possible to obtain, under sterile conditions, varying degrees of pressure and pulse rate. All vessels were perfused with human plasma, and the gas mixture consisted of 75% nitrogen, 20% oxygen, and 5% carbon dioxide.

Since we were interested in studying lipid synthesis as well as cholesterol transfer, we added 2-^{14}C-sodium acetate and cholesterol-1,2-^{3}H (^{3}H-cholesterol) to the perfusion fluid. If synthesis of lipids occurs, then the acetate will be incorporated into lipids. On the other hand, if cholesterol is being transferred in toto into the vascular wall, then ^{3}H-cholesterol can be traced into the vascular wall. The main difficulty at the onset consisted

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of bringing cholesterol into solution. This was finally possible by sonication. The cholesterol was added to a very small amount of concentrated lipid extract from human serum, the mixture was then evaporated in vacuo, and after addition of 5ml of human plasma, the mixture was sonicated three times for 1 minute each at intervals of about 1 minute. Using this procedure, we were able to demonstrate that the cholesterol is bound to the alpha- and beta-lipoprotein fractions of the perfusing serum.

Nicotine (6 mg of nicotine dissolved in 5ml of saline solution) was added prior to adjusting the final volume of 250 ml with human plasma. Nicotine was added to one perfusion system and the other perfusion pump served as control. In the control experiments, saline was used in place of nicotine.

Lipid analysis of the artery was carried out by means of extraction by Folch mixture and, after refluxing, separating it on a thin-layer plate of silica gel according to the method of Freeman and West. After separation of the fractions, they were eluted with eluting solvents, and the activity was counted in a tricarb liquid scintillation spectrometer.

Cholesterol was determined by the method of Zak and coworkers in a Kintrac spectrophotometer. The details of the method are described in our publications (see enclosure). The results on atherosclerotic coronary arteries may be summarized as follows: (1) atherosclerotic human coronary arteries cannot synthesize

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cholesterol from acetate; (2) only very limited synthesis of cholesterol esters from acetate occurs; (3) free cholesterol is taken up by the artery; (4) nicotine has only a very slight effect on lipid synthesis from acetate, it has no effect on free cholesterol uptake. It may be mentioned at this point that in normal coronary arteries, as well as in saphenous veins perfused at either low venous or higher arterial pressure, nicotine had no influence on either synthesis of lipids or on uptake of cholesterol or cholesterol esters.

The experiments on coronary arteries, therefore, demonstrate that human coronary arteries differ in many respects from animal coronary arteries and that many of the data accumulated in the literature on animals do not apply to human vessels. Of prime importance was the finding that cholesterol is not synthesized in the human artery but is passively transferred into the vascular wall, as first suggested by Virchow.

We subsequently started a series of experiments in which we investigated lipid metabolism and the effect of nicotine on perfused human nonatherosclerotic coronary arteries and saphenous veins. As mentioned above, it was difficult to obtain a sufficiently large series of normal human coronary arteries, but there has been no lack of saphenous veins because of the popularity of coronary artery surgery.

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Source: https://www.industrydocuments.ucsf.edu/docs/yfbb0000

The technique used in these experiments was similar to that described above. Saphenous veins were removed from patients prior to performing aortic coronary saphenous grafts. The human coronary arteries were obtained from individuals within 5 hours after death. The oldest of these individuals was 18 years old, most of them being children who had succumbed to malignant lymphomas. Here again, the effect of nicotine on lipid synthesis and cholesterol transfer was studied in human saphenous veins perfused at a pressure of 45/35 mmHg. Nicotine tartrate was added to the perfusion fluid to make a final concentration of 150 μM. We were interested in investigating whether or not the uptake of cholesterol by saphenous veins differed in vessels perfused at low as compared to high pressure. This question is of some importance since in coronary surgery the venous graft is exposed to arterial pressures.

In principle, the results did not differ greatly from those obtained on atherosclerotic human coronary arteries. To summarize, (1) human coronary arteries, without evidence of atherosclerosis, did not differ from atherosclerotic arteries in their inability to synthesize cholesterol from acetate; (2) arteries with and without atherosclerotic lesions take up cholesterol from the perfusion fluid to an equal degree; (3) human saphenous veins perfused at relatively low pressure (45/35 mmHg) do not differ from atherosclerotic and normal coronary arteries in their ability

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Source: https://www.industrydocuments.ucsf.edu/docs/yyd0000

to synthesize lipids; (4) the uptake of cholesterol, however, is less than that of coronary arteries or of saphenous veins perfused at arterial pressures (130/100 mmHg); (5) human saphenous veins perfused at arterial pressures (130/100 mmHg) take up cholesterol from the perfusion fluid equal to that taken up by coronary arteries; (6) in saphenous veins perfused at high pressure, synthesis of lipids from acetate equals that in saphenous veins perfused at lower pressure or of nonatherosclerotic coronary arteries; (7) nicotine failed to influence either synthesis of lipids or uptake of cholesterol.

As to be expected, the uptake of cholesterol was significantly greater in coronary arteries as compared to the saphenous veins perfused at low pressure. This suggests that hemodynamic conditions influence the uptake of cholesterol by the vein from the perfusion fluid; this is in line with the idea of filtration or imbibition of cholesterol. It is also in agreement with the findings of others, such as Werthessen, who found that when calves' aortas were perfused at high perfusion pressure, the amount of cholesterol accumulated by the aorta was limited. The position of cholesterol in areas of the aorta exposed to turbulence is well known. It is believed that the increased uptake of cholesterol in these areas may be the direct result of hemodynamic damage and/or interaction of

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Source: http://www.industrydocuments.ucsf.edu/docs/ytyf0000

foreign elements, particularly platelets with endothelium at points of disturbed flow, as suggested by Packham. The role which permeability of the vascular wall plays in the uptake of cholesterol is not clear, and further experiments are planned to investigate this particular problem. It has been found however, that increased influx of Evan's blue in the intima occurs with increased pressure or wall strain and increased shearing stress; it appears that the increased flux of this dye into the vascular wall parallels augmented uptake of free cholesterol. Possibly, it is the circumferential tension which acts as a determinant of transendothelial passage of proteins by widening the endothelial intercellular spaces through which the transfer can occur.

The finding of increased cholesterol uptake by veins exposed to high perfusion pressure may have significance in determining the eventual fate of aortic coronary saphenous grafts. We believe that this is now being recognized by surgeons, who prefer, if at all possible, an arterial over a vein graft. Finally, the experiments indicate that within the limits of our experiments, nicotine has no effect on the synthesis or the uptake of lipids by human coronary arteries.

It may be seen from the bibliography that a total of 9 papers have been supported by the Council for Tobacco Research during the last year.

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Source: https://www.industrydocuments.ucsf.edu/docs/ytb000

BIBLIOGRAPHY
Supported by the CTR

1. T. Morita, and R.J. Bing: Lipid metabolism in perfused human coronary arteries, Proc. Soc. Exp. Biol. Med., 140:617, 1972.

2. H. Hashimoto, J.S.M. Sarma, J. Mao, E. Larsen, and R.J. Bing: Lipid metabolism in perfused human nonatherosclerotic coronary arteries and saphenous veins. Submitted to Atherosclerosis.

3. R.J. Bing, and K. Hellberg: Measurement of coronary flow in ischemic heart disease (edited by W. Likoff, B.L. Segal, W. Insull, Jr.), in Atherosclerosis and Coronary Heart Disease, page 146, 1972, Grune & Stratton, Inc.

4. R.J. Bing, K. Hellberg, and O. Pachinger: Recognition of coronary artery disease, New York State Journal of Medicine, 72:1818, 1972.

5. G. Stefan, and R.J. Bing: Echocardiographic findings in experimental myocardial infarction of the posterior left ventricular wall, the American Journal of Cardiology, 30:629, 1972.

6. R.J. Bing, and K. Hellberg: Coronary blood flow in relation to angina pectoris, Circulation 46:1146, 1972.

7. O.M. Pachinger, and R.J. Bing: Coronary blood flow assessment with xenon and rubidium. Submitted to Seminars in Nuclear Medicine.

8. R.J. Bing, and K. Hellberg: Pharmacodynamics of the coronary microcirculation (edited by J.P. Davidson), in New Perspectives in Diagnosis and Management of Myocardial Infarction, Williams & Wilkins Co., in press.

9. R.J. Bing, H. Wayland, A. Rickart, and K. Hellberg: Studies on the coronary microcirculation by direct visualization. To be published in the Russian Journal of Cardiology.

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Source: https://www.industrydocuments.ucsf.edu/docs/ytjd0000

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyf0000

THE COUNCIL FOR TOBACCO RESEARCH – U.S.A., INC.

P-5

February 5, 1973

Grant Application No. 844R1

To: The committee comprising Drs. Bing, Cattell and Sommers

Subject: Duane G. Wenzel, Ph.D., University of Kansas, Lawrence
Renewal application No. 844R1
"Nicotine and Carbon Monoxide on Vascular Lipid Disposition"

History

CTR support, which began in 1957, has been interspersed with several denials. The current grant is $15,849.

Support is now requested in the amount of $16,264 (earlier estimate $15,323) for the second and final year of a two year program, to have priority in competition "if progress is satisfactory".

Documents Submitted (attached)

1. Application dated January 23, 1973.

2. Progress report April 1, 1972 - January 15, 1973.

Comment

This grant provides summer salary for Dr. Wenzel.

As page 4 of the application indicates, Wenzel has NIH support for a separate carbon monoxide study.

F.W.N.

FWN:wg
Encls.

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Comm.
Dr. Bing
Dr. Cattell
Dr. Sommers

CARDIOVASCULAR

#844R1
#844-5/1/72-4/30/73
CF #415 & 415A
1/1/65-5/31/70Inc
CF 728 9/69 denied
CF 473 3/65 denied
CF 345 9/1/62-2/28/64
CF 234 7/59 denied
CF 155 7/1/59-8/31/59

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.
110 EAST 59TH STREET
NEW YORK, N. Y. 10022

FEB 1 1973

Application For Renewal of Research Grant

First ☒ Second ☐

Date: January 23, 1973

1. Name of Investigator(s): (include title and degrees) Duane G. Wenzel, Ph.D.
Professor and Chairman
Department of Pharmacology and Toxicology

2. Institution & Address: Department of Pharmacology and Toxicology
School of Pharmacy
University of Kansas
Lawrence, Kansas 66044

3. Short Title of Project: Nicotine and Carbon Monoxide on Vascular Lipid Disposition

4. Proposed Renewal Starting Date: (Anniversary or other) July 1, 1973

5. Discuss any Important Changes or Additions to Objectives or Specific Aims:

The objectives and specific aims remain essentially the same.

6. Give a Brief Statement of your Working Hypothesis if altered or modified:
The working hypothesis has been expanded to include: a) the effects of agents produced in vivo from the CO and (or) nicotine; b) the mechanism of increased membrane lability; and c) a shift of cellular lipids from dispersed to a globular form.

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Source: https://www.industrydocuments.ucsf.edu/docs/yvv0000

11. Budget (for coming year)

A. Salaries (Personnel by names or category)		% time	Amount
Professional			
Duane G. Wenzel	3 mo.	100%	$7298
Fringe benefits FICA, TIAA, HI & UI on principal investigator			560
	9 mo.	10%	(not requested)
Technical			
Research Assistant (Not selected)	12 mo.	50%	3600
		Sub-Total	
B. Consumable Supplies (list by categories)		$11,458	
Culture medium, serum, solvents chemicals, glassware			1000
Animals, feed and supplies			800
		Sub-Total	
C. Other Expenses (itemize)		1,800	
Travel to meeting of Tissue Culture Association			300
Reprints, postage, telephone, books, and journals			150
		Sub-Total	
D. Permanent Equipment (itemize)		450	
Still for double distilled water (replacement)			500
		Sub-Total	
E. Overhead (15% of A+ B +C)		500	
15% of $13,708		2,056	
		Total $16,264	

It is understood that the applicant and institutional officers in applying for a grant have read and found acceptable the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Signature [signature] Director of Project Duane G. Wenzel

(913) 864-4001 Telephone

Signature [signature] Business Officer of the Institution Henry L. Snyder

(913) 864-3126 Telephone

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

7. Changes or Additions to Experimental Design and Procedures: (Attach Separate Pages)

A potentially critical factor in studying the effects of tobacco smoke components on cultured heart cells is that one must consider agents which produce effects in vitro that are equivalent to hypothetically critical in vivo effects. Effects or substances produced in vivo by CO and (or) nicotine, not likely to be seen in vitro, include: lactic acidosis, free fatty acids, and the catecholamines. In addition to CO and nicotine, these agents will now also be tested directly upon the cultured cells. Experiments have been conducted to determine the proper concentrations of the agents to be tested and factors concerned with their addition.

A second modification of the experimental design is one of emphasis. In the original proposal (Section III A 3b) it was proposed that fat inclusions be studied cytochemically via oil red-O staining. It is now proposed, in accord with the hypothesis of Dixon (1970), that one of the earliest signs of a breakdown in normal cell metabolism is a shift of intracellular lipids from a dispersed to a globular state. Such changes are seen in vessel walls in the pre-atherosclerotic state, and in the heart and other organs as a result of diabetes, poisoning by CCl_4 or ethanol, malnutrition, and infections. A reproducible method for quantitating changes in globular lipids has already been developed. Concomittant changes in phospholipid is being studied by use of acid hematein staining. Changes in the form of cholesterol within the cell is being studied by polarization microscopy.

(see page 2a)

8. Additional Requirements:

The only thing requested beyond that anticipated when the project was initiated is a new still for preparing glass redistilled water. The present still is many years old and is subject to frequent down time. It is also located in an area of high contamination yet is too large to be accomodated elsewhere. The sum of $500 is requested to purchase a small (Kontes) glass and teflon still and install it in a more suitable location.

9. Changes in Personnel with Biographical Sketches of new Personnel (append)

None

10. Publications or Papers in Press resulting from the Project or closely related work

Brenner, G.M. and Wenzel, D.G., Carbon monoxide and cultured rat heart cells. I. Inhibition of cell growth and maintenance of beating rate. Toxicol. Appl. Pharmacol. 23, 251-262 (1972).

(see page 3)

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7. Changes or additions to experimental design and procedures (continued):

Effects of hypoxia and of nicotine upon the form of intracellular lipid have already been evaluated. The secondary agents (lactic acid, free fatty acids, and catecholamines) are now being studied.

A third modification of the experimental design concerns the lability of lysosomal and mitochondrial membranes. This approach was initiated because of interest in the potential role of lysosomal enzymes in the effects of smoking. Earlier studies (Wenzel and Richards, 1970) suggested that lysosomal enzymes may be released from the heart during hypoxia and treatment with nicotine. Instead of measuring the changes in a given lysosomal enzyme (β-glucuronidase) as had been proposed (Section III B), a modification of the method of Gomori (1950) for lysosomal staining, was used to determine the lability of the lysosomes. The major difficulty of the method, namely the problem investigators have had in getting reproducible results, was overcome and uniform results can now be obtained. This method has been tested with a variety of labilizing and stabilizing agents. These results are currently being prepared for publication. Further studies have been conducted to develop a method for the measurement of changes in the permeability of the mitochondrial membrane. Studies are currently underway to demonstrate the comparative effects of labilizers on both lysosomal and mitochondrial membranes of heart muscle and endothelial cells.

As soon as the above studies are completed the effects of the substances believed to be secondary factors in the effects of CO and nicotine (lactic acid, free fatty acids, and catecholamines) are to be tested for their effects on the permeability of lysosomal and mitochondrial membranes. These effects will, in turn, be evaluated in terms of the development of globular lipids and cell viability. Once such relationships can be established then potential mechanisms related to lipid synthesis and uptake, and to changes in acid mucopolysaccharides, or to lipoprotein lipase, as originally proposed, will be tested.

References

Gomori, G. Arch. Path. 82, 189 (1941).

Dixon, K.C. Histochem. J. 2: 151 (1970).

Wenzel, D.G. and Richards, M.H. Toxicol. Appl. Pharmacol. 16: 656 (1970).

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10. Publications or papers in press (continued):

Wenzel, D.G. and Brenner, G.M., Carbon monoxide and cultured rat heart cells. II. Interaction of carbon monoxide and hypoxia on growth and contractile activity. Toxicol. Appl. Pharmacol. 24: _____ (1973) In press.

Source: https://www.industrydocuments.ucsf.edu/docs/ytvl0000

Other Sources of Financial Support

List financial support for research from all sources, including own institution, for this and/or related research projects.

Current

Title of Project	Source	Amount	Duration
Carbon Monoxide and Cultured Heart Cells	NIH Environmental Health Sciences	$27,804 $26,063 $26,391	1972-73 1973-74 1974-75

Pending

None

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

CTR-USA Grant #844

Progress Report No. 1

April 1, 1972 to January 15, 1973

Duane G. Wenzel
Professor and Chairman
Department of Pharmacology and Toxicology
School of Pharmacy
University of Kansas
Lawrence, Kansas 66044

NICOTINE AND CARBON MONOXIDE ON VASCULAR LIPID DISPOSITION

Because of the changeover in the anniversary dates of grants, this report covers a period of 9½ months.

The experimental design of the original proposal called for three types of measurements: biochemical, cytochemical, and microscopic (morphologic). The cytochemical and morphologic measurements were the first to be employed. Because of the initial results and their potential relationship to cardiovascular "injury", total effort during this 9½ month period has been a cytochemical-morphologic approach.

1.) Effect of Treatments on Cytoplasmic Lipid Inclusions.

Lipid inclusions in cultured muscle and endothelioid cells were stained with oil-red O, by a modification of the method of Luna (1968). Several methods were tested for the measurement of the amount of stained lipid. These included: assignment of a grade to all of the cells on an entire cover glass; extraction of the oil-red O from stained cells on a cover glass and making a colorimetric measurement; and counting the number of cells with visible (400X) lipid inclusions in 100 cells in each of 5 or 10 randomly-selected fields on a given cover glass. Although the count per 1000 cells was the most time consuming of the methods employed, it was by far the most reproducible and is now routinely employed.

One mechanism by which a change of intracellular lipids from the dispersed to the globular state may occur is through a lack of phospholipid or interference with the dispersing power of phospholipid (Dixon, 1970). For this reason and because an increase in stainable (unmasked) phospholipid is a reliable and sensitive indicator of ischemic myocardial injury (Niles and Barnhouse, 1967; Niles et al., 1968), Baker's (1946) acid hematein method for staining phospholipids was tested on separate cultures in conjunction with oil-red O staining.

Treatments tested to date with these methods include: a cholesterol and (or) β-lipoprotein-enriched medium, several concentrations of nicotine, and a combination of the lipid-enriched media and nicotine. The results of these experiments are still being evaluated.

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Source: https://www.industrydocuments.ucsf.edu/docs/ybyl0000

2.) Lysosomal Lability

We have already demonstrated the presence of lysosomes with acridine orange and fluorescence light in both cultured muscle and endothelial cells from the rat heart. Treatment of these cells with 75% CO for up to 10 days did not appear to alter the distribution or size of the lysosomes, but reduced their number (Brenner and Wenzel, 1972). It has been demonstrated that exposure of cells to hypoxia ($\downarrow O_2$) produces increased permeability of the lysosomes. In the heart, $\downarrow O_2$ produces a marked release of lysosomal enzymes (Brachfeld and Gembra, 1965) and a large shift from bound to the free form (Leighty *et al.*, 1967). In our experience (Wenzel and Richards, 1970), the *in vivo* effect of $\downarrow O_2$ on lysosomal enzymes depends upon not only degree of $\downarrow O_2$ and its duration, but the time after recovery from $\downarrow O_2$ that measurements are taken.

The 'lysosome concept' has been the subject of many reviews (de Duve, 1959; de Reuck and Cameron, 1963; Gahan, 1967; Straus, 1967; Allison, 1968; Dingle and Fell, 1969). The lysosome is a single-membrane-limited organelle which contains a large number of lytic enzymes. Damage to the lysosomal membrane results in the release of these enzymes into the cell. The lysosome contains 36 known hydrolases including 4 phosphatases. One of these enzymes, acid phosphatase, has become a 'marker' enzyme for the lysosome, and lysosomes are frequently characterized by its presence.

Gomori (1950) reported a technique for the histochemical demonstration of acid phosphatase in tissue sections. The Gomori technique uses a suitable organic phosphate as substrate for the phosphatase, and a soluble lead salt to capture and localize the phosphate moiety after it has been removed by the enzyme. The reaction is conducted at pH 5, which is close to the pH optimum for acid phosphatase. The precipitated lead phosphate is later converted to black lead sulfide for visualization of the reaction sites.

In studying acid phosphatase activity, de Duve (1959) found that the substrate beta-glycerophosphate did not readily penetrate the membrane of the intact (or 'latent') lysosome unless the organelle (or cell) has been treated with an agent capable of altering the structure of the lysosomal membrane, and thus the permeability of the membrane to beta-glycerophosphate. Bitensky (1962, 1963a, 1963b) utilized this finding in the development of the so-called 'lysosomal fragility test'. Bitensky reported that unfixed tissue sections required 20 min. incubation in the Gomori medium to yield stained lysosomal particles, whereas shorter incubation times did not result in lysosomal staining. Fixed tissues, on the other hand, were found to stain more rapidly (10 min) due to the increase in permeability of the lysosomal membrane brought about by the process of fixation. The rationale behind the lysosomal fragility test of Bitensky is that cell damage is accompanied by alterations in the permeability of the lysosomal membrane, and that these alterations can be detected before other abnormalities. Bitensky proposed that the lysosomal fragility test could be used to indicate the early stages of cell damage induced by a variety of agents. The fragility test was designed to assess the ability of the lysosomal membrane to withstand a controlled amount of damage (caused by the incubation medium), and thus to infer the condition of the membrane before it was subjected to this damage.

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A number of workers have used the lysosomal fragility test of Bitensky as an index of cellular toxicity (Allison and Mallucci, 1965; Gahan, 1965; Berger and Fand, 1967; Baccino, 1969; Fand, 1970; Gabrielescu, 1971; Réne, Darden and Parker, 1971; and others). All of these workers, however, have used the method in an 'all-or-none' fashion by using only one incubation time, or a small number of incubation times. None of these investigators have attempted to continuously grade their results by following the intensity of the lysosomal staining reaction with increasing time of incubation. Previous workers have also not attempted to evaluate their results statistically.

The modified procedure which we have developed involves direct staining without prior fixation (the lead in the incubation medium acts as a fixative). The coverslips (with the cells attached) are washed briefly in normal saline and transferred to an incubation medium containing 0.01 M sodium-beta-glycerophosphate (Sigma), 0.004 M lead nitrate and 0.05 M sodium chloride in 0.05 M acetate buffer (pH 5.0). The cells are incubated in the medium at 37°C, and two coverslips are removed from the medium at 5 minute intervals. The coverslips are allowed to drain for a few seconds after removal from the incubation medium and excess medium is removed by blotting the edge of each coverslip against absorbent paper. The coverslips are next transferred to hydrogen sulfide-saturated distilled water for exactly 5 min, after which they are rinsed in distilled water for a few minutes, drained, and mounted on microscope slides with Kaiser's glycerol gelatin.

Coverslips are examined by dark-field microscopy (200X). Each coverslip is systematically scanned, and an average assessment of the extent of lysosomal staining is allotted to each. Each coverslip is rated on 2-4 separate occasions on a 'blind' basis. The mean value of the ratings assigned to each incubation time (usually 2 coverslips) is calculated and taken as the "Weighted Average Grade" for that particular incubation time.

The following grading scale is used:

1 - Minimal or non-specific 'background'staining only (including the nuclear membrane and nucleoli).
2 - Lysosomes starting to stain in some cells.
3 - Weak lysosomal staining in most cells (> 90% of cells).
4 - Moderately strong lysosomal staining in most cells (> 90% of cells).
5 - Maximal lysosomal staining in most cells (> 90% of cells).

Color photographs of cells stained for increasing periods are taken and response curves are plotted representing the extent of lysosomal staining (recorded as the "Weighted Average Grade") with increasing incubation time in the beta-glycerophosphate medium.

Preliminary studies indicate the suitability of the above grading scale and the feasibility of this initial set of experiments. After having completed this study of the relationship between the duration of incubation and the change in acid phosphatase staining several other tests to measure the effects of several potential injury factors were evaluated. These include the following:

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differences in culture media, normal saline, isotonic sucrose, hypotonic sucrose, distilled water, acetate buffer, acetic ethanol (5%), formalin (10%), absolute acetone, triton X-100 (0.1% and 0.01%), hydrocortisone (20 and 50 μg/ml), vitamin A (20 μg/ml), chloroquine phosphate (30 and 50 μg/ml), 95% oxygen (from 15 hr to 6 days), hypoxia (from 2 days to 10 days), carbon monoxide, 75% (from 4 to 10 days), carbon monoxide, 95% (from 4 to 10 days), and nicotine (0.5 mM, 1 mM and 2 mM).

Results are now being evaluated via a variety of statistical treatments. It is apparent at this time that the method is reproducible and permits a comparison of labilizing or stabilizing agents on the basis of either dose or duration of treatment. The method continues to be refined and evaluated.

3.) Mitochondrial Lability

In the original proposal the method of Kakari (1970) was proposed as a means for cytochemically detecting early lipid formation within the cell. The method is based upon the staining of minute fat globules near the mitochondria with the formazan (reduced NBT) produced by a nitroblue tetrazolium reaction for dehydrogenase activity. Cultured heart muscle or endothelioid cells treated with a lipid-enriched medium (added cholesterol and β-lipoprotein), stained with increased intensity with the NBT test. However, because the mitochondria and lipid droplets are similar in size and staining characteristics, cells were then extracted with acetone to remove lipid before staining. Although lipids were removed, formazan staining of mitochondria was greatly speeded. Since the presence of formazan-stained bodies does not readily discriminate between mitochondria and lipids but appears to detect increased labilization of the mitochondria, this procedure is being studied as to its suitability for this purpose. Tests are now ongoing to further refine and evaluate the method.

Because mitochondrial lability has been suggested as a factor in the adverse consequences of the release of lysosomal enzymes Emanuelli et al. (1969), studies of CO and nicotine and their in vivo concomitants on lysosomal and mitochondrial lability will include an evaluation of the time-order in which labilities may occur as well as the relative susceptibility of the two cell types being studied.

4.) Ultrastructural Changes

To date the time spent studying cultured muscle and endothelioid cells by electron microscopy has been confined to the methodology by which it is possible to obtain suitable specimens, and to the evaluation of the ultrastructure of these cell types. Work has just now started in an attempt to demonstrate a) ultrasturctural changes in intracellular lipid distribution and b) changes in lysosomes or mitochondria associated with the effects of nicotine and (or) CO and their in vivo concomitants.

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Source: https://www.industrydocuments.ucsf.edu/docs/rjmc0000

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Source: https://www.industrydocuments.ucsf.edu/docs/kyl0000

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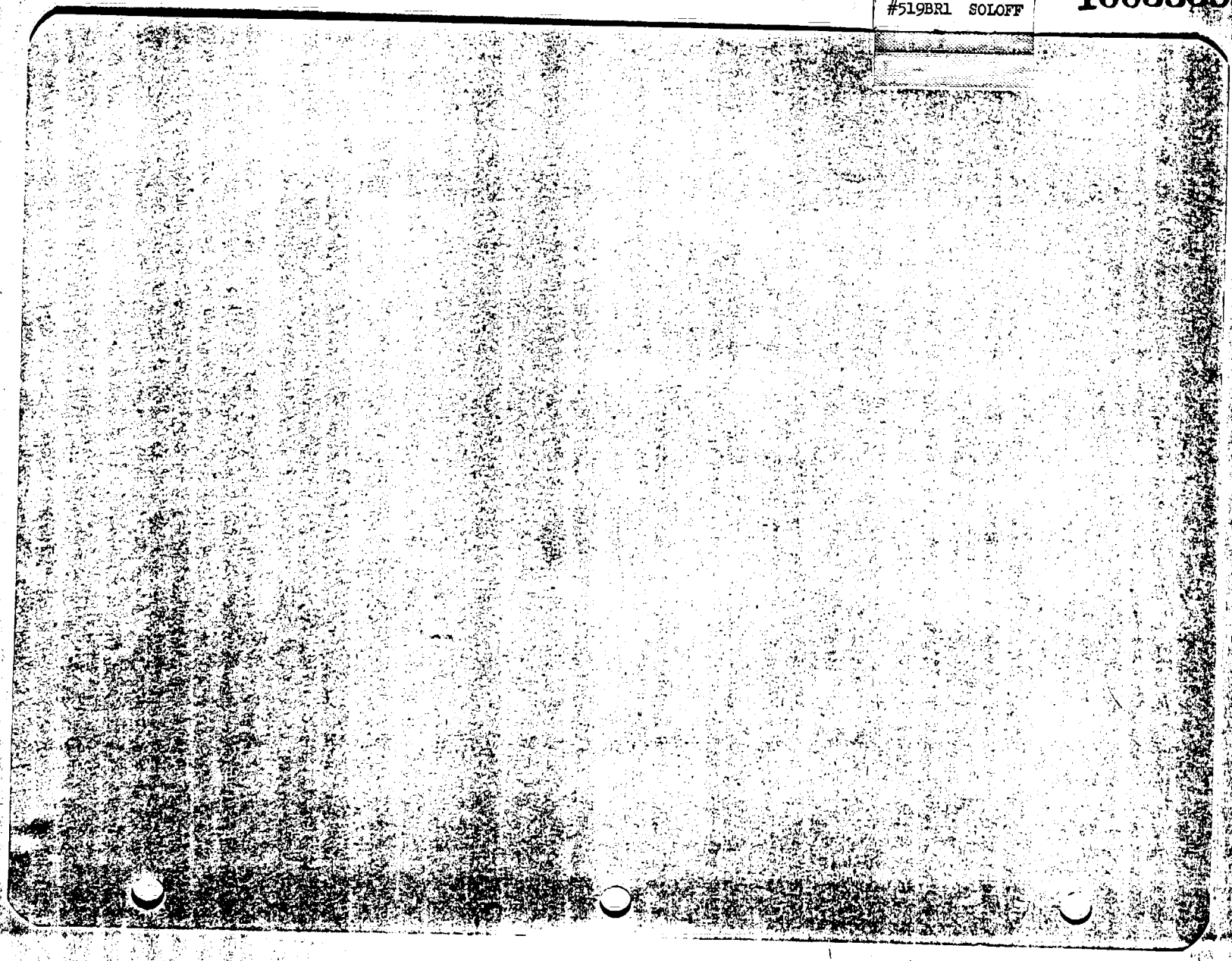

Source: https://www.industrydocuments.ucsf.edu/docs/ymlf0000

THE COUNCIL FOR TOBACCO RESEARCH – U.S.A., INC.

P-5

January 24, 1973

First Renewal Grant Application No. 519BR1

To: The committee comprising Drs. Bing, Cattell and Jacobson

Subject: Louis A. Soloff, M.D., Temple University Health Sciences Center, Philadelphia
First Renewal Application No. 519BR1
"Purification and physiologic significance of lecithin cholesterol acyl transferase (LCAT)"

History

This grantee has been supported since 1963.

Application No. 519BR-1 requests $85,016 (original estimate for this year was $73,539). The letter of award implies that the present application has priority in competition, and "takes note" that the original application proposed a three year project.

Documents Submitted (attached)

1. Application dated January 16, 1973.
2. "Publications in 1972" (2 pages).
3. Curriculum vitae of K.G. Varma Raja, new staff member.

Comment

Enclosed is a copy of Dr. Bing's comment after his site visit on October 19, 1972.

The last progress report received from Dr. Soloff was No. 1 July 1, 1971 - January 31, 1972 and was distributed to you in the March, 1972 SAB agenda book.

F.W.N.

FWN/vr
Encl.

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TEMPLE UNIVERSITY
HEALTH SCIENCES CENTER
PHILADELPHIA, PENNSYLVANIA 19140

January 29, 1973

Dr. Frederich W. Nordsiek
Associate Director
The Council For Tobacco Research - U.S.A., Inc.
110 East 59th Street
New York, N.Y. 10022

Dear Dr. Nordsiek:

I was delighted to have the opportunity of speaking with you over the telephone and hope that sometime in the near future we can meet.

I enclose two copies of our paper entitled "Measurement of the Initial Rate of Serum Cholesterol Esterification" which was accepted for publication by Biochemical Medicine on June 12, 1972. I anticipate that this should be published within the next month or two.

One of the reviewers also wanted an amplification of number 2, under section 5 of our application. Our data have shown, as yet unpublished, that LCAT activity is slightly lower in the female than in the male. We have pursued this finding by measuring LCAT activity throughout three consecutive menstrual periods in the female. This study has been completed but our chemical data are not as yet available because we wanted to do all determinations simultaneously. Hopefully this data will be available soon and ready for submission for publication. We wondered whether the cholesterol metabolism in the ovary might have some bearing on the differences in LCAT activity between male and female and for that reason wanted to determine whether there were any consistent changes throughout the menstrual cycle. PReliminary data suggest that there is such a cyclic change but I am not as yet prepared to say so with certainty.

We have studied LCAT activity both in fasting and fed rat, and incidentally have also studied LCAT activity in several different animals. A paper has already been prepared on LCAT activity in various animals with an attempt to relate LCAT activity to resistance and susceptibility to atherosclerosis. I enclose a copy of this manuscript but would like you and whoever reviews the manuscript to recognize that it is still confidential because it has not been published. Our work on the fasting and fed rat suggest differences from that which has been reported in the literature previously by cruder methods of measuring LCAT but again all of the data have not been collected so that a definitive statement cannot be made as yet.

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We have found some very interesting inter-relationships between post-heparin lipolytic activity and LCAT and a manuscript has been submitted for publication. I enclose this manuscript but again would like to call your attention, and the reviewers',to the fact that this manuscript should be regarded as confidential because the material has as yet not been accepted for publication or published.

We are, at the present time, studying the possibility that the surface of the red blood cell contains LCAT. There is suggestive evidence in our original work on the interaction between the arterial wall and red blood cell which to my mind could not be explained unless there were actually LCAT on the surface of the red blood cell separate and distinct from LCAT in the plasma. These studies are too premature for me to discuss at the present time.

I think you already have abstracts on our work which tends to relate thyroid over and under function to LCAT activity and also the possibility that familial type II hyperlipoprotenemia may be associated with a deficiency of LCAT activity.

These papers are being prepared for publication. We are also studying the possibility that there might be a cholesterol ester hydrolyzing enzyme in plasma and this work whould be completed within the next month or two. Finally, we are fortunate enough to have Dr. Wallace Clark, Professor of Pathology and in charge of electronmicroscopy here at Temple, to study some of the morphologic changes of the lipoproteins after we have stimulated our patients with heparin and other drugs. WE have as yet not started any experiments with respect to smoking.

If there are greater details or you would like preliminary reports on any of these studies which have as yet not been written in final manuscript form, I shall be very happy to send them to you.

Sincerely yours,

Louis A. Soloff, M.D.
Blanche P. Levy Distinguished
Service Professor
Professor of Medicine

LAS:jc
Enclosures

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CARDIOVASCULAR

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A., INC.

COMMITTEE
Dr. Bing
Dr. Cattell
Dr. Jacobson

110 EAST 59TH STREET
NEW YORK, N. Y. 10022

HISTORY
No. 519BR-1
519B
519A 5/1/69-6/1/72
519 2/1/66-2/1/69
Cr 374 2/1/63-2/1/66

Application For Renewal of Research Grant

First [X] Second [ ]

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JAN 2 3 1973

Date: January 16, 1973

1. Name of Investigator(s): (include title and degrees)
Louis A. Soloff, M.D.
Professor of Medicine

2. Institution & Address:
Temple University Health Sciences Center
3400 North Broad Street
Philadelphia, Penna. 19140

3. Short Title of Project:
Purification and physiologic significance of lecithin cholesterol acyl transferase (LCAT).

4. Proposed Renewal Starting Date: (Anniversary or other)
June 1, 1973

5. Discuss any Important Changes or Additions to Objectives or Specific Aims:

Our objective and specific aims have not changed: to increase fundamental information concerning the metabolism of blood cholesterol, which occupies the center of the predominent theory of the pathogenesis of atherosclerosis. Our approaches are 1) to purify lecithin:cholesterol acyl transferase (LCAT), the enzyme responsible for esterification of plasma cholesterol. In the past, the major obstacle to solution of this problem has been instability of the enzyme (half life 3.5 hours) once a certain stage of purification has been achieved. This year we have been able to obtain a preparation which is highly purified and appears stable for weeks or possibly months, a feat, to my knowledge not yet accomplished elsewhere. The preparation is approximately 1500 fold concentrated if LCAT activity divided by the absorption at 280 nm is used as the index of purification. This preparation is obtained after affinity chromatography, and contains one major and about three minor components as judged by disc gel electrophoresis. Preliminary experiments indicate that LCAT activity is associated with the major component. 2) At the same time, we are determining the physiologically important initial rate of esterification of plasma cholesterol in a variety of disorders and changes in this rate produced by physiologic, pharmacologic and hormonal stimuli and also by smoking. Publications listed under (10) represent the types of work we have done in 1972. In addition, manuscripts will soon be prepared on (a) LCAT activity during the menstural cycle. (b) LCAT activity in the fasting and fed rat (c) interactions between post heparin lipolytic activity and LCAT (d) a hypothesis explaining competitive inhibition of free fatty acids and lysolecithin (a product of LCAT) on LCAT activity (e) arterial wall, RBC and plasma contributions to LCAT activity.

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6. Give a Brief Statement of your Working Hypothesis if altered or modified:

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JAN 2 3 1973

Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

7. Changes or Additions to Experimental Design and Procedures: (Attach Separate Pages)

None

8. Additional Requirements:

Gas chromatograph:

At the present time we are using the gas chromatograph in the Clinical Research Center. This apparatus is also being used by four other active research workers so that it is available to us only part-time. Furthermore the Clinical Research Center is on the second floor of the hospital and our laboratory is on the fifth floor of the chemical laboratory building which is on the other side of Broad St. Our own gas chromatograph is over 13 years old and not capable of doing some of the work we are doing and plan to do. Part-time use of the Clinical Center's apparatus has slowed our own work.

9. Changes in Personnel with Biographical Sketches of new Personnel (append)

See attached sheet.

10. Publications or Papers in Press resulting from the Project or closely related work

See attached sheets.

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

R: REDACTED MATERIAL

11. Budget (for coming year)

A. Salaries (Personnel by names or category)	% time	Amount
Professional		
Soloff, Louis A.	30%	
Rutenberg, Harold L.	20%	
Lacko, Andras G.	100%	
Technical		
Baldwin, F.	100%	
Nickols, P.	100%	
Raja, K.G. Varma	100%	
Sub-Total		
Fringe Benefits 12%		
B. Consumable Supplies (list by categories)		55536.00
Chemicals, radiochemicals, glassware, vials, caps, service, plasma volunteers, etc.		7,000
Heavy Water		4,000
Sub-Total		11,000
C. Other Expenses (itemize)		
Travel for three professionals @ $500 each		1,500
Secretarial assistance		500
Sub-Total		2,000
D. Permanent Equipment (itemize)		
Gas Chromatograph		8,500
		75,036
E. Overhead (15% of A+ B +C)		9,980
Total		85,016

REDACTED

It is understood that the applicant and institutional officers in applying for a grant have read and found acceptable the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Signature _____
Director of Project

Telephone

Signature _____
Business Officer of the Institution

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Telephone

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

Other Sources of Financial Support

List financial support for research from all sources, including own institution, for this and/or related research projects.

Current	Title of Project	Source	Amount	Duration ending
	The money for the research laboratory building (in which we work) was supplied by a general grant from the United States Public Health Service.			

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

Publications in 1972

1. On the rate of cholesterol esterification in cord blood serum. Lacko, A.G., Rutenberg, H.L. and Soloff, L.A. Lipids 7:426, 1972.

2. In vitro serum cholesterol esterification in coronary artery disease. Rutenberg, H.L., Stern, A.G., Soloff, L.A. and Braverman, S. de. Amer. Ht. J. 84:348, 1972.

3. Recovery of labeled cholesterol and cholesterol esters from thin layer chromatograms. Lacko, A.G., Rutenberg, H.L., and Soloff, L.A. Clinica. Chim. Acta 39:506, 1972.

4. Serum cholesterol esterification in patients with coronary heart disease: Importance of initial rate of esterification expressed as a function of free cholesterol. Soloff, L.A., Lacko, A.G. and Rutenberg, H.L. Am. Ht. J. (In Press).

5. Measurement of the initial rate of serum cholesterol esterification. Lacko, A.G., Rutenberg, H.L. and Soloff, L.A. Biochem. Med. (In Press).

6. Abstracts:

Reduced rate of plasma cholesterol esterification in patients with coronary heart disease. Lacko, A.G., Rutenberg, H.L. and Soloff, L.A. Fed. Proc. 31:291, 1972.

Esterification of serum cholesterol in hyperthyroidism and hypothyroidism Soloff, L.A., Lacko, A.G., and Rutenberg, H.L. Circ. 46: Suppl. II, pg. 224, 1972.

Esterification of serum cholesterol in familial hyperbetalipoproteinemia Circ. 46: Suppl. II, pg. 224, 1972.

Submitted for publication:

1. Initial fractional rate of esterification of serum cholesterol in familial hyperbetalipoproteinemia. Soloff, L.A., Lacko, A.G., and Rutenberg, H.L.

2. Serum cholesterol esterification in species susceptible and resistent to atherosclerosis. Lacko, A.G., Rutenberg, H.L. and Soloff, L.A.

Unrelated subjects supported by the Council for Tobacco Research, U.S.A.

1. Asymptomatic rapid ectopic tachycardia induced by and persisting during exertion. Rutenberg, H.L. and Soloff, L.A. Amer. J. Card. 29:408, 1972.

2. Clinical significance of the angiographic demonstration of coronary atherosclerosis. L. A. Soloff Amer. Ht. J. 83:727, 1972.

3. On the electrocardiogram and clinical improvement of myocardial aneurysmectomy Soloff, L.A. and Rutenberg, H.L. Amer. Ht. J. 83:237, 1972.

1003539279

Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

4. Cardiac deterioration replacing cardiac pain after surgery to revascularize the heart. L.A. Soloff Amer. Ht. J. 84:446, 1972.

5. An assessment of human cardiac transplantation. Fadali, M. and Soloff, L.A. Amer. Ht. J. (In Press).

Books:

1. Coronary Heart Disease and Coronary Arteriography in Coronary Heart Disease. Grune and Stratton (In Press).

2. Parasystole in Cardiac Arrhythmias. Grune and Stratton (In Press).

10035392S0

Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

R: REDACTED MATERIAL

CURRICULUM VITAE

K.G. Varma Raja

REDACTED

Office address: Lipid Research Laboratory, Temple University Health Sciences Center, 3401 North Broad Street, Philadelphia, Penna. 19140

Home address: REDACTED

Degrees:
-B.Sc. Madras University, Madras, India. Major-Chemistry minors-Physics and Mathematics. REDACTED
-M.Sc. University of Baroda, Baroda, India. Major-Biochemistry, minors-General Chemistry and Physiology.
-Ph.D. University of Illinois, Illinois, U.S.A. REDACTED

Appointments:
1949-50 School teacher, St. Thereas's High School, Trichur.
1950-52 Demonstrator, St. Joseph's College, Bangalore.
1952-56 Demonstrator, Zamorines College, Calicut. India.
1956-58 Research Associate, Fisheries Technological Station Madras, India.
1958-60 Research Assistant, T.B. Chemotherapic Center, Madras India.
1967-68 Research Associate, Biology Department, University of Colorado, Boulder, Colorado.
1968-69 Visiting Asst. Professor, Biology Department, University of Colorado.
1969-70 Visiting Asst. Professor and Research Associate, Johns Hopkins University, Baltimore, Maryland.
1970-72 Research Associate Hebrew Geriatric Center and Hospital, Baltimore, Maryland. Associate, Environmental Medicine, Johns Hopkins Hospital, Baltimore, Maryland.

Membership: REDACTED

Fellowship: Wright Fellow, University of Illinois 1963-66.

Publications:
K.G. Varma: Effect of para aminosalysilic acid on the loss of acid fastness in tubercle bacillus by isoniacid. Ind. J. Med. Res. 49:194, 1961.
K.G. Varma and S.P. Mistry: Incorporation of glycine intchuclaic acid purines of biotin deficient rats. Fed. Proceedings 25: 784, 1966.
K.G. Varma and S.P. Mistry: A possible physiological role of pyruvate carboxylase in the nitrogen sparing action of carbohydrates. Fed. Proceedings 26:286, 1967.
Tepper, B.S. and K. G. Varma: Metabolism properties of purified suspensions of M. lepramurium. 73:143, 1972. J. Gen. Microbiol.

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Huntington Memorial Hospital

100 CONGRESS STREET · PASADENA, CALIFORNIA 91105
TELEPHONE (213) 796-0381

October 19, 1972
Site Visit
Dr. Soloff

We are all familiar with Dr. Soloff's study dealing with the enzyme lecithin cholesterol acetylase. Actually his work is not very much concerned with smoking, but it is excellent work with a meticulous methodology dealing with all aspects of the enzyme. The ramification of this work, of course, is considerable as far as lipid chemistry is concerned. It is highly sophisticated.

Dr. Soloff has applied for a grant from the Public Health Service for $40,000, which he says is inadequate to fund the laboratory, and he needs our money badly. He feels that he can do much more in this field.

I feel the work is excellent although it is not directly related to smoking. The Council should discuss and suggest different studies by this excellent investigator.

Richard J. Bing, M.D.

RJB:bb

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PULMONARY

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

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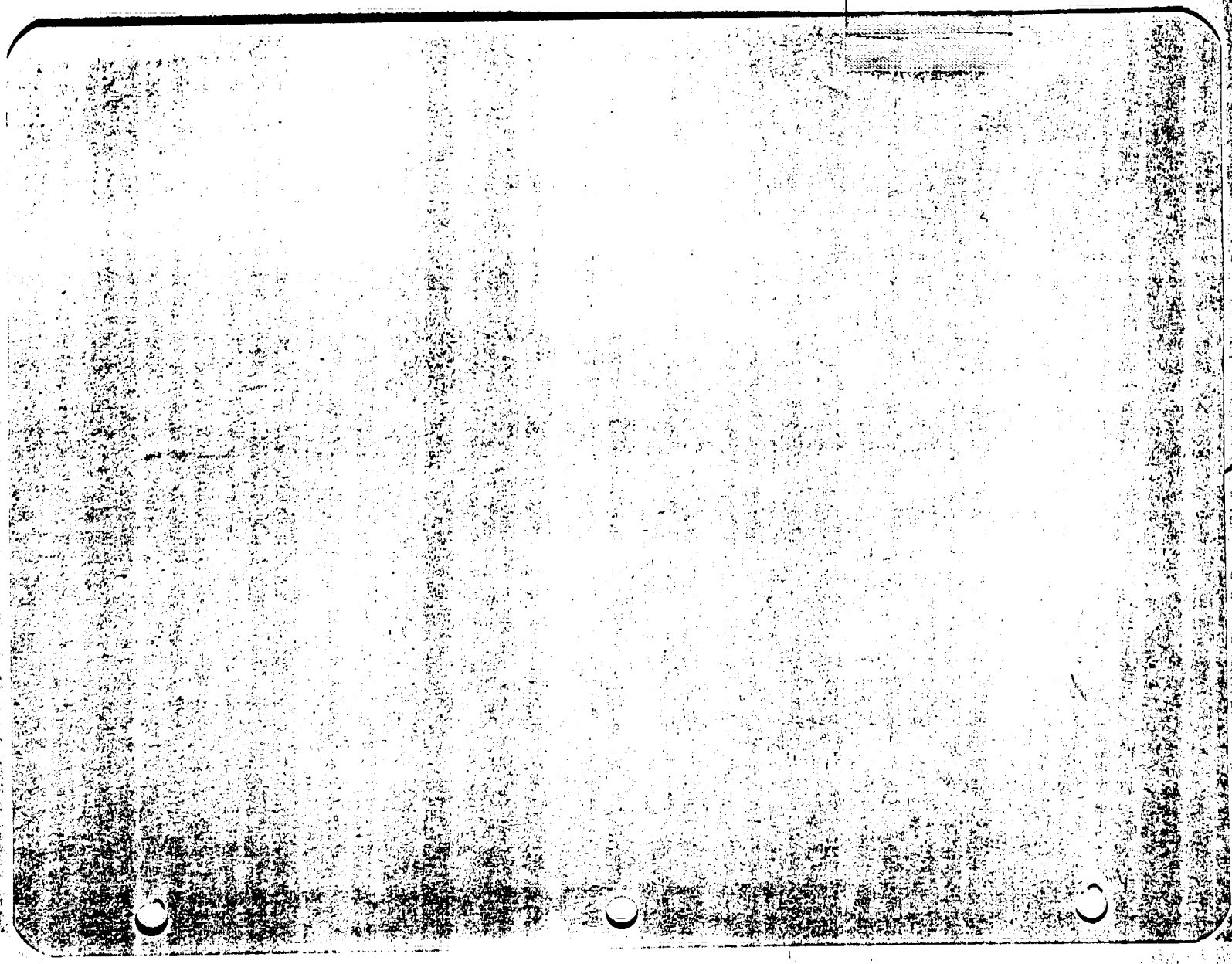

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THE COUNCIL FOR TOBACCO RESEARCH – U.S.A., INC.

January 30, 1973

P-2

Grant Application No. 808R2

To: The committee comprising Drs. Gardner, Loosli, and Sommers

Subject: Allen B. Cohen, M.D., Ph.D., University of California, San Francisco
Renewal application No. 808R2
"Human Alveolar Macrophages and Emphysema"

History

This program began in 1971. The enclosed application requests support for the third and final year of a three year program voted priority in competition.

The request is for $12,487. (The current grant is $16,407).

Documents Submitted (attached)

1. Application dated January 17, 1973.
2. Progress report #2, 10/15/71 - 12/31/72.

F.W.N.

FWN:wg
Encls.

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Comm.
Dr. Gardner
Dr. Loosli
Dr. Sommers

CHRONIC PULMONARY DISEASES

#808R2
#808R1-4/1/72-3/31/73
#808-4/1/71-3/31/72

THE COUNCIL FOR TOBACCO RESEARCH—U.S.A., INC.

110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

Application For Renewal of Research Grant
(Use extra pages as needed)

First Renewal ☐ Second Renewal ☒

Date: Jan. 17, 1973

RECEIVED
JAN 29 1973

1. Principal Investigator (give title and degrees):

Allen Barry Cohen, M.D., Ph.D., Assistant Professor of Medicine

2. Institution & address:

University of California Service
San Francisco General Hospital
1001 Potrero Street
San Francisco, California 94110

3. Department(s) where research will be done or collaboration provided:

Department of Medicine

4. Short title of study:

Human Alveolar Macrophages and Emphysema

5. Proposed renewal date: April 1, 1973

6. How results to date have changed earlier specific research aims:

No changes since 1972 renewal application.

7. How results to date have changed earlier working hypothesis:

No changes.

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8. Any additional facilities now required? Describe briefly:

No increased facilities needed.

Please see attached page for details of additional equipment needs.

9. Any changes in personnel? Append biographical sketches of new key professional personnel:

No changes in professional personnel.

Laboratory technician added in April, 1972: Ralph Knight,
BS biochemistry, Washington University, Seattle.
Two years experience in Lab of Neurath and Wilcox.

10. Append outline of experimental protocol for ensuing year.

11. List publications or papers in press resulting from this or closely related work. (append reprints or manuscripts not previously sent).

see attached list

12. Summary progress report (append in standard form as separate document, unless recently submitted)

10035392S2

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13. Budget for the coming year:

A. Salaries (give names or state "to be recruited")	% time	Amount
Professional (give % time of investigator(s) even if no salary requested)		
Allen B. Cohen	35	-0-
Technical		
Ralph Knight	50	-0-
Sub-Total for A		-0-

B. Consumable supplies (by major categories)

Animals for antisera - $1000

Chemicals including enzymes and substrates - $2000

Small miscellaneous equipment and consumable supplies - $2000

Sub-Total for B $5,000

C. Other expenses (itemize)

Travel (one Eastern meeting)	$500
Publication costs	$250
Equipment maintenance	$1000

Sub-Total for C $1,750

Running Total of A + B + C $6,750

D. Permanent equipment (itemize)

pH Stat - Radiometer

Sub-Total for D $4,724

E. Indirect costs (15% of A+B+C) E $1,013

Total request $12,487

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

14. Other sources of financial support:

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Alpha-1-antitrypsin: Does it inhibit an enzyme which causes emphysema?	National Heart and Lung Institute, NIH (SCOR) HL 14201	34,117	12/1/72 to 11/30/73

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
NONE			

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name Allen B. Cohen, M.D., Ph.D.

Signature Allen B. Cohen Date 11/1/73

Telephone 415 (Area Code) 648-8200 (Number) 273 or 627 (Extension)

Checks payable to

Regents of the University of Calif.

Mailing address for check:

1487 Fourth Avenue

San Francisco, Calif. 94122

Responsible officer of institution

Typed Name Leona M. Butler

Title Gifts and Endowments Officer

Signature _____ Date _____

Telephone 415 (Area Code) 666-2047 (Number) (Extension)

10035392269

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8. Additional Requirements:

While the spectrophotometer has filled our needs for enzymes and alpha-1-antitrypsin analysis to date, it has severe limitations because only substrates which change their optical absorbance spectrum can be used. A full examination of the human alveolar macrophage enzyme inhibited by alpha-1-antitrypsin will necessitate an exploration of its actions against selected artificial substrates. This work requires a pH stat.

In addition, our investigations on the biochemical mechanism of alpha-1-antitrypsin (also credited to 808R) are now in a phase which requires examination of enzyme kinetics for artificial substrates in the presence of chromagenic reagents. Such kinetic analysis can only be done with a pH stat.

I am, therefore, requesting a pH stat for the 03 year of 808R. Since my research technician previously funded on 808R is now funded on the NIH grant, the total amount of funds requested will approximate the amount requested in the initial application. If the salary savings ($1,800) from the late hiring of Mr. Knight in the 02 year of 808R can be carried to the 03 year, the requested budget will not exceed needs projected in the initial application.

pH Stat - Radiometer Components

III - pH meter and controller		$1,138
PHA - 943 Titrigraph module		298
SBR 3		1,260
ABU 12-T with Burette B 220		821
Burette B230		108
Thermal jacket V 526		42
Console D 764		48
TTA - 31 Titration Assemblies		596
PHA 940 Two channel titrator module		166
	Subtotal	$4,477
5.5% State Sales Tax		247
	Total	$4,724

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PART I: Protocol for continued investigations of enzymes which cause emphysema in alpha-1-antitrypsin deficient subjects.

I am continuing my efforts to localize enzymes in lung which bind to alpha-1-antitrypsin. Antibodies to alpha-1-antitrypsin are being labeled with rhodamine in order to search for enzymes in lung which bind to alpha-1-antitrypsin.

In recent months an abstract was published by Galdston (1) which indicated that elastase is deficient in neutrophils from patients who have a deficiency of alpha-1-antitrypsin and do not have emphysema. This may be an important observation. If a genetic deficiency in neutrophil elastase, by itself, prevents the emphysema from developing in patients deficient in alpha-1-antitrypsin, then it is reasonable to assume that the missing elastase contributes a major share of the lung damage which develops in patients deficient in alpha-1-antitrypsin. If the observation can be confirmed, one might suspect that an inhibitor of this enzyme would prevent the emphysema which these patients develop. It is, therefore, important for other laboratories to confirm Galdston's observation. Since the original protocol was designed to identify enzymes which could cause emphysema in patients deficient in alpha-1-antitrypsin, I will test Galdston's observation in two siblings who have Pi Type ZZ deficiency in alpha-1-antitrypsin and no evidence of emphysema.

PART II: Protocol for continuation of the study of the biochemical mechanism of alpha-1-antitrypsin.

Niecamp (2) has used a polyacrylamide gel electrophoretic technique to determine the conditions which will provide maximal shift of enzyme inhibitor complex toward the free enzyme and altered inhibitor with a cleaved peptide bound at the carboxyl side of the inhibitory site. I will try to establish these conditions for alpha-1-antitrypsin. The amino acid which can then be released by carboxypeptidase (3) will then indicate the amino acid at the inhibitory site of alpha-1-antitrypsin which binds to each enzyme. If these data establish that all enzymes act at a limited number of amino acid sites in a manner similar to the manner that enzymes bind to amino acids in their substrates, then I can begin to develop inhibitors which act on the same enzymes that alpha-1-antitrypsin acts on.

References:

1. Galdston, M., C. Gottwalt, A.L. Davis, and A. Janoff: Fed.Proc. 31:282, 1972.

2. Niecamp, C., H. Hixson and M. Laskowski: Biochem. 8:16, 1969.

3. Ozawa, K. and M. Laskowski: J. Biol. Chem. 241:3955, 1966.

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Source: https://www.industrydocuments.ucsf.edu/docs/ylyl0000

11. Publications or papers in press resulting from this or closely related work:

1. Cohen, A.B. and Cline, M.J.: The human alveolar macrophage: Isolation cultivation in vitro and studies of morphologic functional characteristics. J. Clin. Invest. 50:1390, 1971.

2. Mann, P., Cohen, A.B., Finley, T., and Ladman, J.: Alveolar macrophages: Structural and functional differences between nonsmokers and smokers of marijuana and tobacco. Lab. Invest. 25:111, 1971.

3. Murray, J.F., Cohen, A.B., Gold, W., and Greenspan, R.: Early diagnosis of obstructive lung disease. Calif. Med. 116:37, 1972.

4. Cohen, A.B.: The human alveolar macrophage: Isolation, cultivation in vitro and studies of morphologic and functional characteristics. Dissertation for Doctor of Philosophy. December, 1972.

5. Cohen, A.B. and Cline, M.J.: In vitro studies of the foamy macrophage of postobstructive endogenous lipoid pneumonia in man. Am. Rev. Resp. Dis. 106:69, 1972.

6. Cohen, A.B., and Cline, M.J.: Human alveolar macrophages: Functional differences between smokers and nonsmokers. Abstract Clin. Res. 18:483, 1970.

7. Cohen, A.B.: A new hypothesis for the mechanism of action of alpha-1-antitrypsin and supporting experiments. Clin. Res. 20:575, 1972 *

8. Cohen, A.B.: Biochemical mechanism of action of alpha-1-antitrypsin. Submitted to J. Biol. Chem. 1972

9. Cohen, A.B.: Interrelationships between human alveolar macrophages and alpha-1-antitrypsin. Submitted to J. Clin. Invest. 1973.

10. Cohen, A.B.: Purification of two populations of human alveolar macrophages. Submitted to Am. Rev. Resp. Dis. 1973.

* This work received the Cecile Lehman Mayer Research Award for basic sciences of pulmonary diseases in 1972 by the American College of Chest Physicians.

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Source: https://www.industrydocuments.ucsf.edu/docs/

Mailing address:
Chest Service
San Francisco General Hospital
San Francisco, Calif. 94110

CTR # 808R
Report # 2
10/15/71-12/31/72

PROGRESS REPORT

Human Alveolar Macrophages and Emphysema

Allen B. Cohen, M.D., Ph.D.
University of California, San Francisco

PART I: Enzymes in man inhibited by alpha-1-antitrypsin

The goal of Part I is to locate enzymes in man which are inhibited by alpha-1-antitrypsin (AAT) and which may therefore produce emphysema in patients deficient in the inhibitor. The first part of the project was designed to investigate the human alveolar macrophage (AM) as a possible source of these enzymes. AM were examined first as a possible source of the causative enzyme(s) because they reside at the site of damage in emphysema.

AM were lavaged from lungs of subjects with normal lung function or from surgical specimens. Proteolytic activity was measured in cytosol by the method of Anson (1). Fluorescent antibody studies were performed by the indirect method of Weller and Coon (2) to determine whether AAT was present in AM.

AM extracts contain maximal protease activity at pH 3.0 (range 2.0-6.0) (fig. 1). The protease(s) is inhibited by purified AAT when measured at pH 4.0.

Fluorescent antibody studies showed that there is AAT in AM. The fluorescence was blocked with unlabeled anti-rabbit gamma globulin and did not occur when cells were treated with anit-human albumin or with normal rabbit gamma globulin instead of anti-AAT. Fluorescence of AM from one patient with normal lung function and a deficiency of AAT (Pi Type ZZ) was minimal, but when the cells were incubated with normal serum before staining, the fluorescence was enhanced.

These studies demonstrate the presence of AAT-inhibitable proteases in AM, and show that AM can concentrate AAT from fluid-phase. Although the relationships between AM protease(s) and AAT require further exploration, AM could provide a source of the protease activity that engenders the emphysema of AAT-deficient subjects.

The studies on AM were facilitated by the development of improved methods for purifying AM from surgical specimens. Two methods of purification were tried. Sedimentation at 1 x g of fluid recovered from lung lavages was followed by erythrocyte lysis. The populations of AM which resulted were more than 96% pure by morphologic and phagocytic criteria. This new method permitted me to employ large numbers of replicates in studies of macrophage

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cathepsins. In addition, sedimentation through a dense aqueous solution of sodium diatrizoate and ficoll (density 1.07 gm/ml) resulted in a band of cells containing 66% (about a 10 fold purification) of foamy macrophages similar to those I have previously found in lungs distal to obstructed bronchi (3). Large amounts of surface active material were documented in this fraction.

PART II: Biochemistry of alpha-1-antitrypsin

Earlier progress reports provided evidence that trypsin and chymotrypsin compete for active sites on alpha-1-antitrypsin (AAT). A recent experiment has allowed me to split the trypsin and chymotrypsin inhibiting functions of AAT thereby proving that the inhibiting sites are overlapping but not identical.

Phenylglyoxal hydrate has been shown to block arginyl residues as long as the protein is maintained in an environment which contains an excess of the blocking reagent (4). Therefore, the following experiment was performed: Phenylglyoxal hydrate was added to AAT. After a suitable incubation period the treated and control samples of AAT were passed through identical sephadex G25 columns to remove the unbound reagent, and the inhibitor was immediately tested for anti-tryptic and anti-chymotryptic activity. The samples were then incubated at 37° C for 48 hours while being dialyzed against buffer. These conditions are sufficient to remove the phenylglyoxal hydrate from the arginyl residues. After dialysis the samples were again tested for anti-tryptic and anti-chymotryptic activity.

The results showed that blocking arginyl residues eliminated anti-tryptic but not anti-chymotryptic activity and the anti-tryptic activity was restored when arginyl residues were unblocked (Table 1). These results improve on my earlier arginine blocking experiments in two ways:

1. The mild unblocking conditions allow regeneration of activity. These results prove that the blocking reagent did not denature the AAT.

2. Splitting of anti-tryptic and anti-chymotryptic functions by blocking arginyl residues confirms that there are two overlapping inhibitory sites on AAT and suggests that the mechanism of inhibition is strongly related to the amino acid specificity of the inhibited enzyme.

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Source: https://www.industrydocuments.ucsf.edu/docs/nyh0000

References:

1. Anson, M.L.: J. Gen. Physiol. 20:565, 1936.

2. Weller, T.H., and A.H.Coons: Proc. Soc. Exp. Biol. Med. 86:789, 1954.

3. Cohen, A.B. and M.J. Cline: Am. Rev. Resp. Dis. 106:69, 1972.

4. Takahashi, K.: J. Biol. Chem. 243:6171, 1968.

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Source: https://www.industrydocuments.ucsf.edu/docs/yjgb0000

Figure

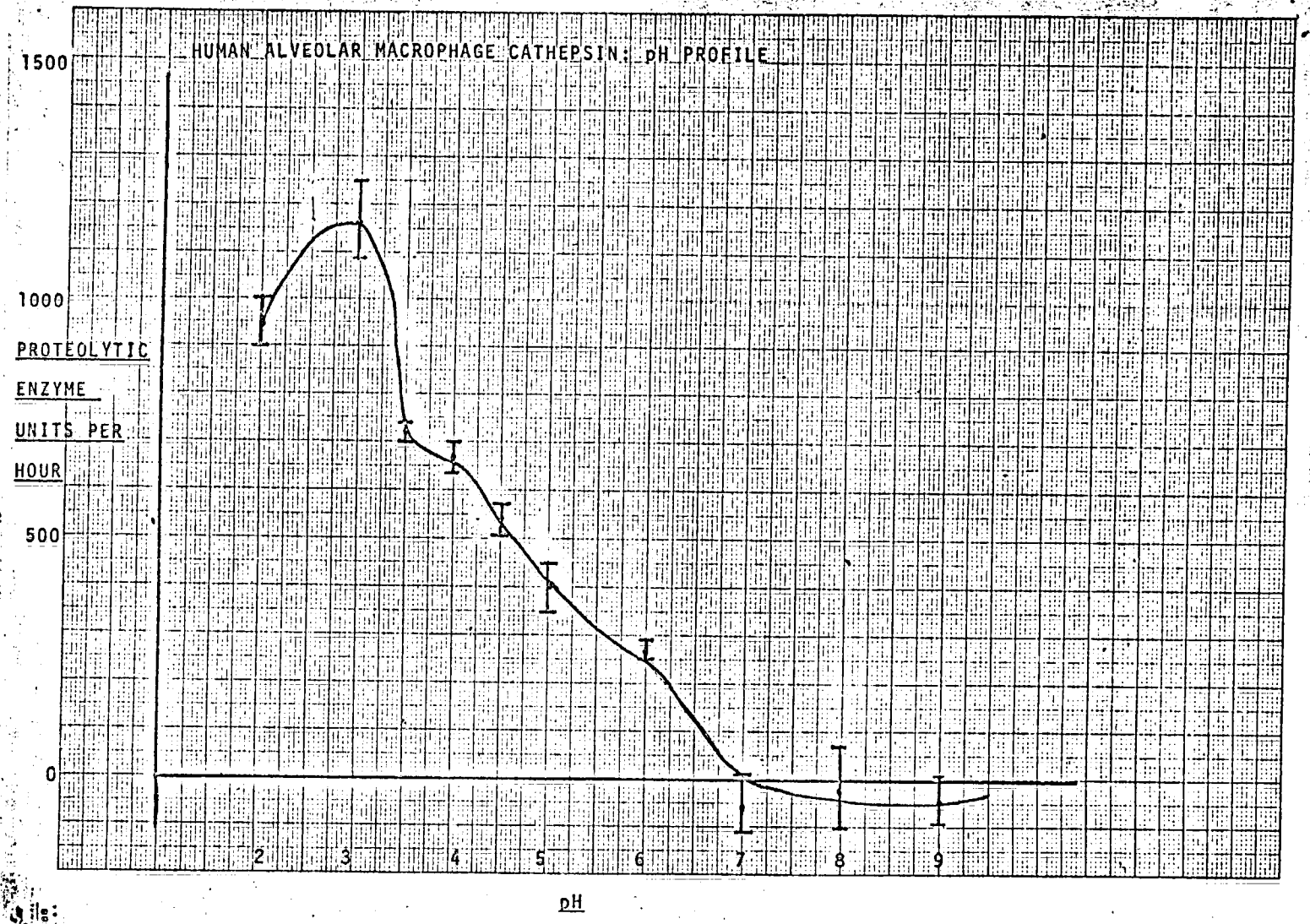

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TABLE I

Effect of Modification of Arginyl Residues on α-1-Antitrypsin Activity

	Enzyme	α-1-Antitrypsin + phenylglyoxal hydrate + enzyme	% Inhibition of enzyme by modified α-1-antitrypsin	α-1-Antitrypsin + enzyme	% Inhibition of enzyme by unmodified α-1-antitrypsin
BAPNA (trypsin substrate)					
1. Mean ΔOD/30 min	.368	.433	0*	.278	24.3†
S.E.	.008	.004		.013	
2. Mean ΔOD/30 min	.337	.347	0*	.236	29.9†
S.E.	.003	.008		.009	
BTEE (chymotrypsin substrate)					
1. Mean ΔOD/min	.069	.043	37.7‡	.051	26.1†
S.E.	.008	.002		.002	
2. Mean ΔOD/min	.064	.040	36.7†	.049	23.4†
S.E.	.001	.000		.000	
BAPNA-After PGH Removal					
1. Mean ΔOD/30 min	.447	.324	27.5†	.309	30.9†
S.E.	.010	.004		.002	
2. Mean ΔOD/30 min	.437	.238	45.5†	.324	27.5†
S.E.	.019	.006		.013	

Levels of statistical significance using Student's t-test:

*>0.2, †<0.001, ‡<0.025

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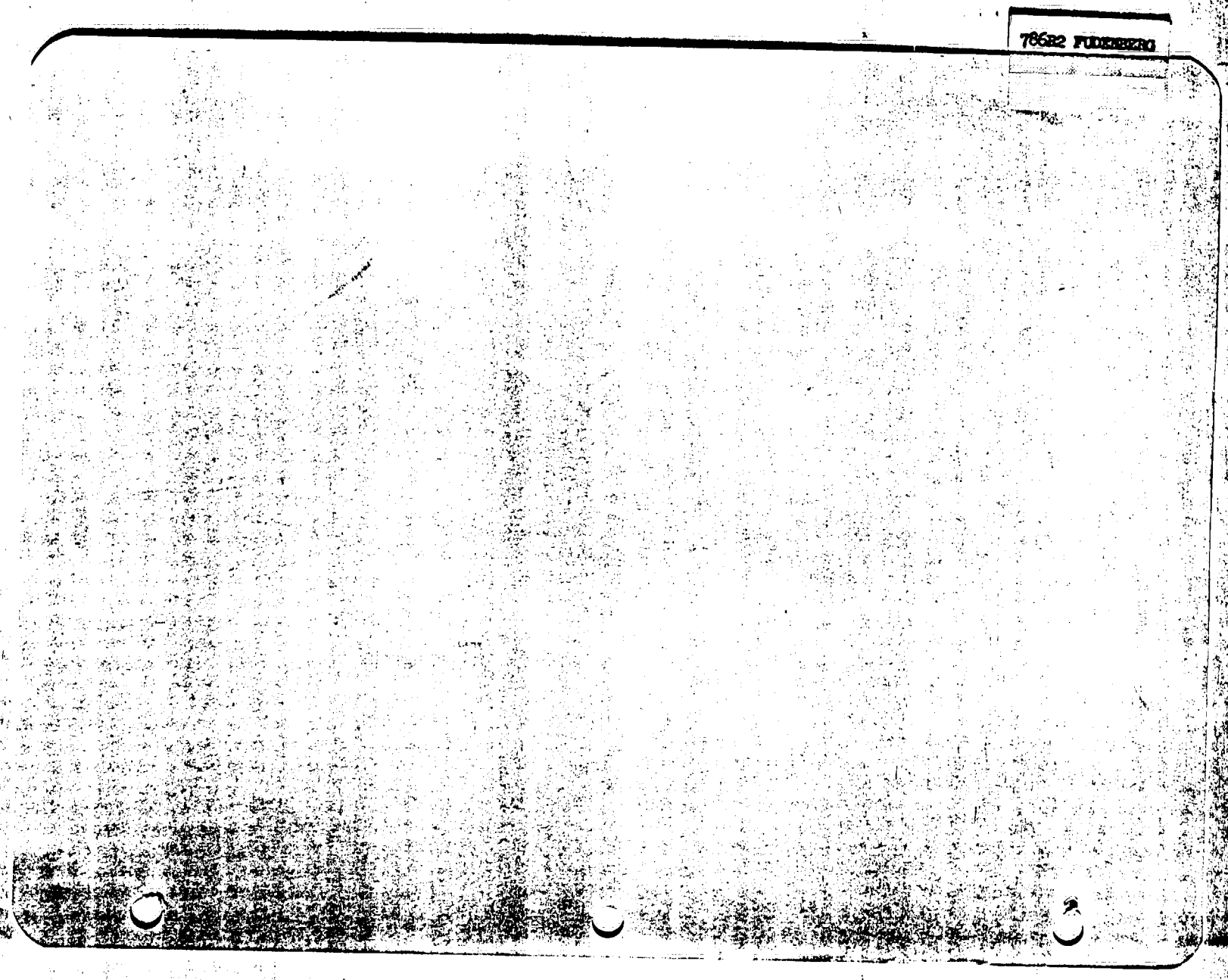

https://www.industrydocuments.ucsf.edu/docs/Atyl0000

THE COUNCIL FOR TOBACCO RESEARCH – U.S.A., INC.

February 7, 1973

P-4

Grant Application No. 786R2

To: The committee comprising Drs. Loosli, Meier and Wyatt

Subject: H. Hugh Fudenberg, M.D., University of California, San Francisco
Renewal application No. 786R2
"Collagen Antibodies in Relation to the Etiology of Emphysema"

History

This request, in the amount of $61,737, is for the third year of an approved three year program. The initial estimate for this year was "about" $42,000.

The current grant is for $59,233.

Documents Submitted (attached)

1. Application dated January 22, 1973, including biographies of Dr. Belton and Dr. Caldwell.

2. Progress report, April 1, 1972 - March 30, 1973.

3. Manuscript "The Freeze-Etch Structure . . .", Belton, Michaeli and Fudenberg, in press, J. Ultrastruct. Res.

F.W.N.

FWN:wg

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UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

BERKELEY • DAVIS • IRVINE • LOS ANGELES • RIVERSIDE • SAN DIEGO • SAN FRANCISCO

SANTA BARBARA • SANTA CRUZ

SCHOOL OF MEDICINE
DEPARTMENT OF MEDICINE

SAN FRANCISCO, CALIFORNIA 94122

January 23, 1972

Robert C. Hockett, Ph.D.
Associate Scientific Director
The Council for Tobacco Research U.S.A., Inc.
110 East 59th Street
New York, New York 10022

Re: #786 Collagen Antibodies in Relation to the Etiology of Emphysema - Council for Tobacco Research, USA

Dear Doctor Hockett:

Enclosed please find fifteen copies of our application for research grant, referenced above.

You will note that salaries for personnel are slightly higher this year. Salaries for faculty and professional research personnel are based on the current July 1972 State appropriations. A yearly merit increase of 5% and a 5% range adjustment is reflected in the salaries of nonacademic personnel.

Sincerely,

H. Hugh Fudenberg, M.D.
Professor of Medicine, UC San Francisco
Professor of Bacteriology and Immunology,
UC, Berkeley

HHF:ljb
Encl.-15 copies

10035630

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THE COUNCIL FOR TOBACCO RESEARCH–U.S.A., INC.
110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

Application for Research Grant
(Use extra pages as needed)

Date: January 22,19

1. Principal Investigator (give title and degrees):

H. Hugh Fudenberg, M.D.
Professor of Medicine, UC San Francisco
Professor of Bacteriology and Immunology, UC, Berkeley

D. Michaeli, Ph.D. (co-Investigator)
Assistant Professor of Biochemistry, UC, San Francisco

2. Institution & address:

University of California
Section of Hematology and Immunology
475 HSW
Third and Parnassus Streets
San Francisco, California 94122

3. Department(s) where research will be done or collaboration provided:

Section of Hematology and Immunology - Department of Medicine

4. Short title of study:

COLLAGEN ANTIBODIES IN RELATION TO THE ETIOLOGY OF EMPHYSEMA

5. Proposed starting date: March 31, 1973 (renewal date)

6. Estimated time to complete: 3 years

7. Brief description of specific research aims:

1. To ascertain the incidence of antibodies to collagen in the sera of smokers and non-smokers with chronic obstructive pulmonary disease (COPD) in control populations matched for age, sex, ethnic origin and smoking history. 2. To investigate the incidence of such antibodies in asymptomatic first-degree relatives of patients with COPD to see if such antibodies indicate genetic predisposition of this disease. 3. To see whether such antibodies bind to lung tissues of affected individuals, thus indicating that they may be a cause rather than a consequence of the disease. 4. To study certain lysosomal enzymes in pulmonary macrophages of patients with COPD and controls (other pulmonary diseases), especially cathepsin D&E, which cleave collagen. 5. To ascertain whether, and if so which, components of cigarette smoke bind to collagen to produce an immunogenic carrier-hapten complex. 6. To determine the three dimensional structure of normal human lung collagen and compare it with collagen from emphysematous lungs.

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8. Brief statement of working hypothesis:

We have found anticollagen antibodies in a high percentage of patients with chronic obstructive pulmonary disease. Whether these are the cause of the disease, that is bicollagen breakdown, whether they arise due to damaged collagen or whether they indicate genetic predisposition to the disease is uncertain. Family studies of the three dimensional normal collagen and of collagen in which antibody is fixed in vivo should help answer this question.

Since macrophages are involved in the first stages of immunologic reactions and since macrophages contain enzymes which probably degrade lung collagen, studies of macrophages of smokers and nonsmokers with or without emphysema, including various enzyme levels, and the correlation with the presence or absence of antibodies will shed additional light on the role of macrophages in the etiology of emphysema.

9. Details of experimental design and procedures (append extra pages as necessary)

Please see attached pages.

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10. Space and facilities available (when elsewhere than item 2 indicates, state location):

2400 square feet - 495 HSW UC Medical Center
1000 square feet - 839 HSE UC Medical Center

Approximately 2,400 square feet of laboratory space is available with cold room facilities, fraction collectors, starch gel, acrylamide gel and immunoelectrophoresis equipment. A Spinco Model 120 C automatic amino-acid analyzer equipped with automatic sample injector and integrator, a Model E analytical ulracentrifuge and equipment for paper chromatography are also available in our laboratory, and we have a separate room for high voltage paper electrophoresis (with proper precautions to avoid laboratory accidents). A Spinco Model 890 protein sequencer is also in use.

11. Additional facilities required:

none. Salaries for faculty and professional research personnel are based on the current July 1972 salary appropriations. A yearly merit increase of 5% and a 5% range adjustment is also reflected in the salaries of nonacademic personnel.

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12. Biographical sketches of investigator(s) and other professional personnel (append): Dr. Pinto da Silva and Dr. Melvin Schanfield are no longer listed on this grant. Dr. John Belton and Dr. J. L. Caldwell have been added. Biographical sketches of both doctors are attached.

13. Publications (five most recent and pertinent of investigator(s); append list, and provide reprints if available).

See references attached to progress report. Copies of "The Freeze-Etch Structure of Lung and Tendon Collagen in Young Rabbits [illegible]

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9A. METHODS;

1. Immunochemical Studies on Collagen:

Polypeptide chains and β components of collagen will be separated by chromatography on CM-cellulose as described by Piez et al. (1). The separated chains will be treated with cyanogen bromide* and the resulting peptides will be separated on phosphocellulose (2). The advantage of this procedure over proteolytic digestion of the polypeptide chains is that, in addition to isolation and identification of the determinants, it will be possible to determine their location in the chain since the order of the CNBr fragments of several collagens has recently been determined (3). The following procedures will be used for assay of antigenic activity of α chains and β components as well as of CNBr fragments of chains:

(a) Precipitin reaction, as described by Michaeli et al. (4), will be used for assays of α and β chains.

(b) Hemagglutination and hemagglutination-inhibition, using the chromic chloride system of Gold and Fudenberg (5) will be used for assay of antigenic activity of the CNBr peptides.

(c) Collagen will be labeled with H^3-glycine by incorporation of the radioactive amino acid in the diet and the labeled chains of collagen will be treated with CNBr as described above. The small peptides, (M. V. 1500-5000), derived from the amino terminal end of the α chains will be assayed by equilibrium dialysis--a technique that will provide quantitative information both on the amount of antibodies directed to a specific peptide and on their binding constants with the peptides. Large, non-dialyzable peptides, will be assayed by the ammonium sulphate precipitation technique (6) yielding essentially the same type of information with regard to amounts of antibodies and estimates of binding constants.

Free collagen and collagen breakdown produced in the serum will be assayed by inhibition of precipitation of radiolabeled antigen

* Hereafter, cyanogen bromide will be abbreviated as CNBr.

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using the method of Levin and Fudenberg (7). The amino acid sequence of peptides possessing antigenic activity will be determined by the Edman setpwise cleavage from the NH_2-terminal end of the peptide as modified by Crestfield et al. (8). Degradations of the carboxy-terminal end will be carried out with carboxypeptidase A and B. Intermediate peptides will be assayed for immunological activity. Immunologically active peptides will be synthesized by the solid phase peptide synthesis (9). The synthetic peptides will be assayed as described above. Also, analogues of the peptides will be synthesized and assayed for immunological activity in order to ascertain the relationship between structure and antigenic activity. Collagen may be converted from a relatively poor antigenic entity into a potent antigen by attachment of foreign chemical groups on it. The presence of anti-collagen activity in sera of some emphysema patients (Fudenberg, and Michaeli, in preparation) suggests that such a mechanism may be operative in this condition. (Identification of the chemical entities in the gaseous phase of cigarette smoke that have the capacity to bind to lung collagen may prove essential in the understanding of the etiology of this disease.)

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2. Serologic Studies:

Sera will be obtained from 300 patients with COPD, as defined by pulmonary function tests (Appendix 1), and from 300 normal control patients matched for age, sex, ethnic origin, and smoking history. Sera will also be obtained from 300 patients with other forms of pulmonary disease though obtaining matching sera by the parameter outlined above may not be feasible. Sera of first degree relatives on whom pulmonary function studies have been done will also be tested.

These sera will be assayed via human collagen and altered collagen, and altered peptides by the chromic chloride hemagglutination method of Gold and Fudenberg (5). The γ-globulins will be isolated from selected sero-positive (and control sero-negative) sera and Fab (antigen-binding) and Fc fragments produced by papain digestion to insure that the antigen-binding is indeed via the Fab fragment (the portion of the molecule which binds to antigen)

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rather than being nonspecific. The whole sera, IgG, and Fab fragments will also be reacted with a synthetic peptide of charg closely similar to collagen, namely poly tyrosyl-glutamyl-argunyl lysine (T, G, A, L) to insure that the reaction occurring is indeed immunologic and not merely due to protein - protei interaction on a "charge" basis.

3. Cellular Immunity Studies:

In the past few years, it has been evident that response of immunologically competent cells (i.e., immune response at the cellular level) is a better parometer of immunologic reactivity to various antigen than is serum antibodies. We will measure cellular immune response to collagen, to modified collagen, and to collagen peptides (in addition to serum antibody) by the following parometers of cellular immunity:

A) Lymphocyte response to antigen, as measured by incorporation of labeled processor, (Usually H^3 - thymidine into DNA) using a synchronized cell in with incorporation of same (10).

B) Hemolysis in gel of red cells coated with antigen by single lymphocytes or plasma cells in the presence of complement, the so-called "Jerne plaque technique" (11).

C) Inhibition of migration in glass by normal macrophages in the presence of both antigen and lymphocyte from sensitized donor - e.g., migration inhibitory factor (MIF) (12).

(The MIF studies will be performed by Dr. Levin, the lymphocyte studies by Dr. Fudenberg, and the Jerne plaque studies by Dr. Henry.)

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4. Electron Microscopic Studies:

After reduction, pepsin fragments of IgG anti-collagen and anti-ferritin will be prepared, antibodies to collagen will be hybridized (13a) with antibodies to ferritin and the hybrid antibody used in attempts to demonstrate collagen antibodies fixing to lung tissue by immuno electron microscopy (13b).

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4.(b) We also propose to study human lung collagen, using the freeze fracture technique, from normal and emphysemous lung in the same way we have studied rat tendon and lung collagen to establish the normal and three dimensional structure in emphysema and the deviations from it, if any.

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5. Pulmonary Macrophage Studies:

Pulmonary macrophage obtained by bronchial aspiration from, patients with COPD and from controls with other pulmonary disease (donors will be obtained during bronchial aspiration and lavage; lysosomal enzyme levels determined by classic chromic assays (14). Special attention will be paid to cathepsin D & E, since evidence exist that these digest collagen (15,16); other lysosomal enzymes (e.g., RNAase, acid phosphotase, etc.) will be measured as controls, especially lysozyme, has anti bacterial properties. (Dr. Hanes, who has had extensive experience with lysosomal enzyme purification and preparation in quantitive measurements will perform these measurements.) Levels of lysosomal enzymes which cleave mucopolysaccharides, (1) Hyaluronidase, (2) β-Glucoronidase, (3) Sialidase, (4) β-Galactosidase, α-Galactosidase, and (5) μ-Acetyl - β-Glucosaminadase, (17) will be measured in view of the obvious relevance to the intracellular cement substance in the lung.

B. SIGNIFICANCE:

1. Antibodies to Collagen:

With regard to the anti-collagen antibodies as possible laboratory markers of genetic susceptibility to development of COPD analogies exist with other antibodies; antibodies to collagen may be consequence, cause, or merely laboratory markers of genetic susceptibility to development of COPD.

Release of collagen into the induction of antibodies to denatured lung collagen may be incidental to the destruction of the lung parenchyma. This possibility is not very likely in view of the low immunogenicity of collagen and the massive doses of collagen required for induction of anti-collagen production (18).

Formaldehyde, which is present in the gaseous phase of cigarette smoke (19), may react with collagen and introduce an increased amount of intermolecular cross-linking. This would have the double-effect of lowered permiability of alveolar walls and increased antigenicity of the collagen. Other compounds in the gaseous phase of cigarette smoke (acetaldehyde, ammonia, acrolein) may react with collagen. Such an interaction may convert collagen from a relatively poor antigenic entity into a potent antigen (20).

Insofar as being laboratory markers of genetic predisposition to COPD, the analogy of anti-collagen antibodies to other antibodies is pertinent. For example, in asymptomatic relatives of patients with rheumatoid arthritis, there is a high incidence

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of antibodies to γ-globulin (rheumatoid factors) of the same type that is present in the vast majority (about 85%) of patients with rheumatoid arthritis. Thus, it appears that the rheumatoid factor is a genetically determined serologic marker capable of detecting that proportion of the population which is genetically predisposed to develop rheumatoid arthritis upon exposure to certain bacterial, viral or other environmental agents (21).

If anti-collagen antibodies are found to be present in high frequency in asymptomatic relatives of patients with COPD, this would provide evidence of a genetic mechanism analogous to the rheumatoid factors. If so, asymptomatic individuals with such antibodies should be discouraged from smoking.

If the antibodies are restricted to the patients and not found in asymptomatic relatives, the question as to whether they are cause or effect then becomes relevant. Demonstration of fixation of such antibodies to lung tissue of patients with COPD but not to similar tissue of patients with other types of lung disease would provide evidence for a pathogenetic role for such antibodies.

Thus, an extensive survey of emphysema patients for anti-collagen antibodies and also titers and of controls matched according to sex, age and smoking habits appears warranted. If a high incidence of such antibodies is indeed present in COPD, we will isolate and identify the antigenic determinants of lung collagen as outlined in "Methods". Furthermore, we will ascertain whether or not lung collagen from emphysema patients is bound by haptenic material. For elucidating this problem, a variety of approaches will be utilized, e.g., measurements of free ε amino groups in collagen from normal and emphysematous lungs, assay of bound formaldehyde in lung collagen, assay of acetyl groups in lung collagen, etc., as outlined in "Methods".

2. Enzyme Studies:

Since parenchymal tissues and alveolar wall of the lungs contain large amounts of collagen, and, since cathepsin D & E may cleave collagen (22), it would be of considerable interest to see if cathepsin levels are increased in pulmonary macrophages of subjects with COPD. Further, cathepsin degrades other tissue protein which may be involved in the integrity of the lung. It has been shown that collagenase secreted extracellularly breaks up long collagen fibers which are then taken into phagosomes for digestion (22). Further, collagen fibers have been identified within lysosomes and digestion of collagen confirmed (23). The mucopolysaccharides are the intracellular cement of the lung

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tissue. Increase in certain lysosomal enzymes in macrophages of COPD would theoretically aid in destructive tissue and development of emphysema.

Of the known lysosomal enzymes, the following cleave mucopolysaccarides (24,25):

1) Hyaluronidase

2) β-Glucoronidase

3) Sialidase

4) β-Galactosidase, α-Galactosidase

5) μ-Acetyl - β-Glucosaminadase

Hyaluronidase is known to be present in macrophages (25), as is β-Glucoronidase. The others have not been specifically looked for in macrophages but are probably present.

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R: REDACTED MATERIAL

FEB. 72

BIOGRAPHICAL SKETCH
of
Dr. John C. Belton, M.S., Ph.D.

Personal:

Name -- JOHN CLAIR BELTON

Place and Date of Birth -

General Health -

Marital Status

Children -

REDACTED

Educational Data:

Undergraduate training in biological science was obtained at Lewis and Clark College in Portland, Oregon.

I spent the year at Colorado State University School of Veterinary Medicine.

Work was completed for a Master of Science degree at Oregon State University with a major in zoology.

Graduate studies leading to a Doctor of Philosophy degree were continued concurrently with a teaching assistantship. This degree was completed in June of 1966 and emphasized cell morphology and physiology.

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Professional Employment:

1956-1957 -- Undergraduate laboratory assistant: Drosophila genetics, Bacteriology, etc.

1959-1964 -- Graduate teaching assistant for courses in general zoology, histology, cell biology, experimental embryology, genetics microtechnique.

1962-1964 -- I produced and organized teaching materials used in general zoology and histology laboratories. This job required a broad understanding of biological photography and microtechnique as well as teaching experience.

1964-1965 -- Instructor at Oregon State University, involved with the coordination of all general zoology laboratories for more than six hundred students. Duties also included specimen preparation, equipment maintenance and production of teaching materials.

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Professional Employment: (Continued)

1966-1969 -- Assistant Professor, California State College, Hayward, I have organized courses in comparative vertebrate histology, cell physiology and microtechnique. I have taught a graduate course on cell differentiation and morphogenesis

1969-1970-- Research Associate (Department of Botany, University of California, Berkeley). I held a research grant from the California Department of Public Health for the study of changes in lung fine structure resulting from exposure to nitrogen dioxide. This research was done in collaboration with Dr. Daniel Branton and provided one year training in the freeze-etch technique.

1970- Present Associate Professor, California State College. Hayward. I have been active in directing graduate student research.

1973 - Assistant Research Immunol.,Lecturer, UC,San Francisco Pending

Research Interests and Publications:

Belton, John C. and Alfred Owczarzak (1968). Cellular changes associated with the pre-ovulatory deposition and storage of hepatic lipids in the frog Ascaphus truei. Herpetologica 24: 113-127.

Belton, John C., D. Branton, H. V. Thomas and P. K. Mueller. (1971) Freeze-etch observation of Rat Lung. Anatomical Record.

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Belton, John C. and Carol M. Belton (1970). Morphogenesis of Larval Integument of Acanthatrium oregonense. Second International Congress of Parasitologists, Washington, D. C.

Belton, J. C., McKally, and Fudenberg, H. H. (Submitted) Freeze-etch structure of lung collagen in young rats. J. Ultrastr. Res.

Belton, J. C., Tueller, E. E., McLaughlin, R. F. and Fudenberg, H. H. (submitted) Morphological changes in collagen associated with early human emphysema. Arch. of Path.

Professional Societies and honors:

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Other:

Transcripts and other information will be furnished upon request.

DR. JOHN C. BELTON
Department of Biology
California State College
Hayward, California 94542

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January, 1973

CURRICULUM VITAE

Joseph Lee Caldwell, M.D.

Birthdate: REDACTED

Education:

B.S.;M.D.	Arkansas State University, Jonesboro, Arkansas, University of Arkansas School of Medicine, Little Rock,	REDACTED

Training:

Intern	St. John's Hospital, Tulsa, Oklahoma, 1966-1967	
Resident	University of Iowa Hospital, Iowa City, Iowa, 1969-1971	
Research Fellow	University of Iowa and Veterans Administration Hospitals, 1971	

Positions:

United States Air Force Medical Officer Arizona and Viet Nam	1967-1969
University of Illinois, Dept. of Biochemistry Fellow	1972-1973
University of California, San Francisco	To be Appointed

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14. First year budget:

A. Salaries (give names or state "to be recruited") Professional (give % time of investigator(s) even if no salary requested)	% time	Amount
R. McLaughlin, Jr., M.D. Res. Assoc.	20%	
John Belton, Ph.D., Asst. Res. Immunol. I	50%	
D. Hanes, Asst. Res. Immunol. I	50%	REDACTED
J. L. Caldwell, Asst. Res. Immunol. I	40%	
C. Henry, Assoc. Res. Immunol. I	25%	
Technical		
D. Aiken, Staff. Res. Associate II	100%	
N. Crise, Staff Res. Associate I	50%	REDACTED
M. Kay, Staff Res. Associate I	25%	
Sub-Total for A		REDACTED
B. Consumable supplies (by major categories)		
Glassware and Chemicals		2,000
Sub-Total for B		2,000
C. Other expenses (itemize)		
Computer programming and multivariant analysis		6,000
Sub-Total for C		6,000
Running Total of A + B + C		53,684
D. Permanent equipment (itemize)		
Sub-Total for D		-0-
E. Indirect costs (15% of A+B+C)		8,053
Total request		61,737

15. Estimated future requirements

	Salaries	Consumable Suppl.	Other. Expenses	Permanent Equip	Indirect Costs	Total
Year 2						
Year 3						

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16. Other sources of financial support:
List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
ALPHA, ANTI-TRYPSIN AND O OTHER ALPHA-GLOBULIN LEVELS IN SMOKERS AND NONSMOKERS WITH AND WITHOUT EMPHYSEMA	American Medical Association Educational and Research Foundation	$143,687 (total for 3 years)	5-1-71--4/30/74

This grant deals with α anti-trypsin genetic variants, α chymotrypsin levels, and other protease inhibitors in relation to the etiology of emphysema. It does not explore the immunologic approach at all. Except for the use of the same patient population, the AMA-ERF study and the Council for Tobacco Research study do not overlap in any way.

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name H. H. Fudenberg, M.D.

Signature [redacted] Date

Telephone [redacted] (Area Code / Number / Extension)

Checks payable to

Reagents, University of California

Mailing address for checks

Gifts and Endowments
1487 4th Avenue
San Francisco, California 94122

Responsible officer of institution

Typed Name Mrs. Leona M. Butler

Title Contracts & Grants Officer

Signature Leona M. Butler Date

LEONA M. BUTLER

Telephone [redacted]

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PROGRESS REPORT No. 2

April 1, 1972-
March 30, 1973

COLLAGEN ANTIBODIES IN RELATION TO THE ETIOLOGY OF EMPHYSEMA

During the period covered in this progress report, research the following problems have been carried out.

I. A detailed study on the ultrastructure of collagen fibers in lung has been completed.

II. A model system for emphysema induced in experimental animals has been developed in our laboratories, and ultrastructural alteration of lung collagen in these models is currently being studied.

III. The reactivity of chemicals present in cigarette smoke with lung collagen is currently being studied.

IV. A method for the extraction and purification of lung collagen has been devised in our laboratories allowing detailed chemical analysis of amino acids and carbohydrate components of this collagen.

V. A study on the immunological characteristics of normal lung collagen and of lung collagen reacted with chemicals present in cigarette smoke has been initiated.

VI. A survey of anti-collagen titers in emphysema patients and the normal population has been continued and expanded.

DETAILED SUMMARY REPORT

I. The freeze fracture technique was used to study the fine structure of collagen from rat tendon and lung. The collagen filaments were organized into a spiraled lamellar substructure within each fibril. Filamentous connections were shown which span the inter-fibriller matrix and unite all of the fibrils into a reticular network. These connections had a range in diameter between 70 to 100 Å. The fibrils were coated with material that was greatly hydrated *in vivo*. On the basis of these observations, we have proposed a new structural model for the collagen fibril. A manuscript has been submitted to the Journal of Ultrastructural Research (1), and another is in preparation.

II. We have observed that chronic administration of high doses of β aminopropionitrile induces changes in the lung of experimental animals that are histologically similar to the changes observed in emphysema. These included breakdown of alveolar septae, atrophy of capillaries and general loss of elasticity of the lung. We are currently engaged in

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examining these changes on the ultrastructural level. We are also studying the immune response of these animals to their lung components, especially lung collagen.

III. Studies on the interaction between acetaldehyde and lung collagen have been initiated. Preliminary results indicate that ε amino groups of lysine residue in the collagen molecule interact with this chemical. We are planning to use radioactive acetaldehyde to determine:

a) The quantitative aspects of this interaction.

b) The location on the molecule of acetaldehyde binding.

c) To determine whether acetaldehyde becomes and antigenic determinant following its interaction with collagen.

We are also planning to examine the ultrastructural effects of acetaldehyde binding to collagen.

IV. Although minute amounts of collagen could be obtained from lung using the classical methods of extraction, we could not obtain sufficient material for detailed chemical and immunological studies. Therefore, it was essential to develop an extraction technique that will increase the yield of this collagen. We found that limited digestion with trypsin at 10°C for 24 hours resulted in a substantial increase in the solubility of lung collagen. This collagen is currently being subjected to the following analysis:

a) Chromatography on CM cellulose columns to separate the polypeptide chains of collagen.

b) CNBr digestion of the isolated chains and chromatography on phosphocellulose and CM cellulose columns. This will allow the rapid determination of any sequence differences between lung collagen and skin-type collagen.

c) The content and composition of the carbohydrate moiety of this collagen is being determined. Also, the availability of large amounts of lung collagen has enabled us to inject rabbits with this collagen at high enough doses to obtain high titers of antibodies.

V. In addition to the above mentioned immunization with normal collagen, we are in the process of preparing large enough quantitites of collagen reacted with acetaldehyde in order to determine whether such an interaction induced immunological changes in the collagen. Specifically, we will test for new determinants which are an integral part of the collagen molecule as well as the possibility that

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acetaldehyde constitutes a major new determinant.

VI. A substantial sample of emphysema patients and normal population has been assayed for anti-collagen antibodies. The results indicate significant increased incidence and titer of anti-collagen in emphysema patients. The results are currently being statistically evaluated and will be published shortly (2).

VII. Methods to test the cellular response of emphysema patients to a specific antigenic determinant to human lung collagen by in vitro addition of the small peptide possessing highest antigenic activity to lymphocytes of patients with emphysema in vitro are awaiting isolation of the small peptide

VIII. In terms of macrophages an animal model for macrophage activity has been developed depending upon the production of a microsomal enzyme, hemoxygenase, by macrophages after ingestion of antigen antibody complexes. The rate of production of the enzyme has the characteristics of an induced enzyme and is dissociated from phagocytosis in that there is a lag phase, a hydrocortisone supressed enzyme induction but this inhibitory effect could be reversed by the addition of glucose and insulin to a culture medium. The initial findings were done with macrophages obtained from rat peritoneal cavity, but similar findings have been obtained with rabbit alveolar macrophages (3).

IX. In preparation for looking at pulmonary alveolar tissue by freeze etch electron mechanism, pulmonary studies using the technique were performed with erythrocyte membrane. One published paper demonstrated the translational movement along the the plane of the human erythrocyte ghost of the membrane particles exposed by freeze-fracture. The membrane particles can be aggregated by incubation of the ghosts in media with a pH in the vicinity of 5.5 or 3.5. The particles are disaggregated in neutral and alkaline media (pH 9.5) and also at pH 4.5. Aggregation of the particles is reversible, prevented by prefixation in glutaraldehyde and by media of high ionic strength. Particle aggregation occurs within two to four minutes. These results are consistent with the concept that the erythrocyte ghost membrane is a planar fluid domain formed by a bilayer membrane continuum which is interrupted by localized, yet mobile, proteic intercalations (4).

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547CR2 GUARNERI

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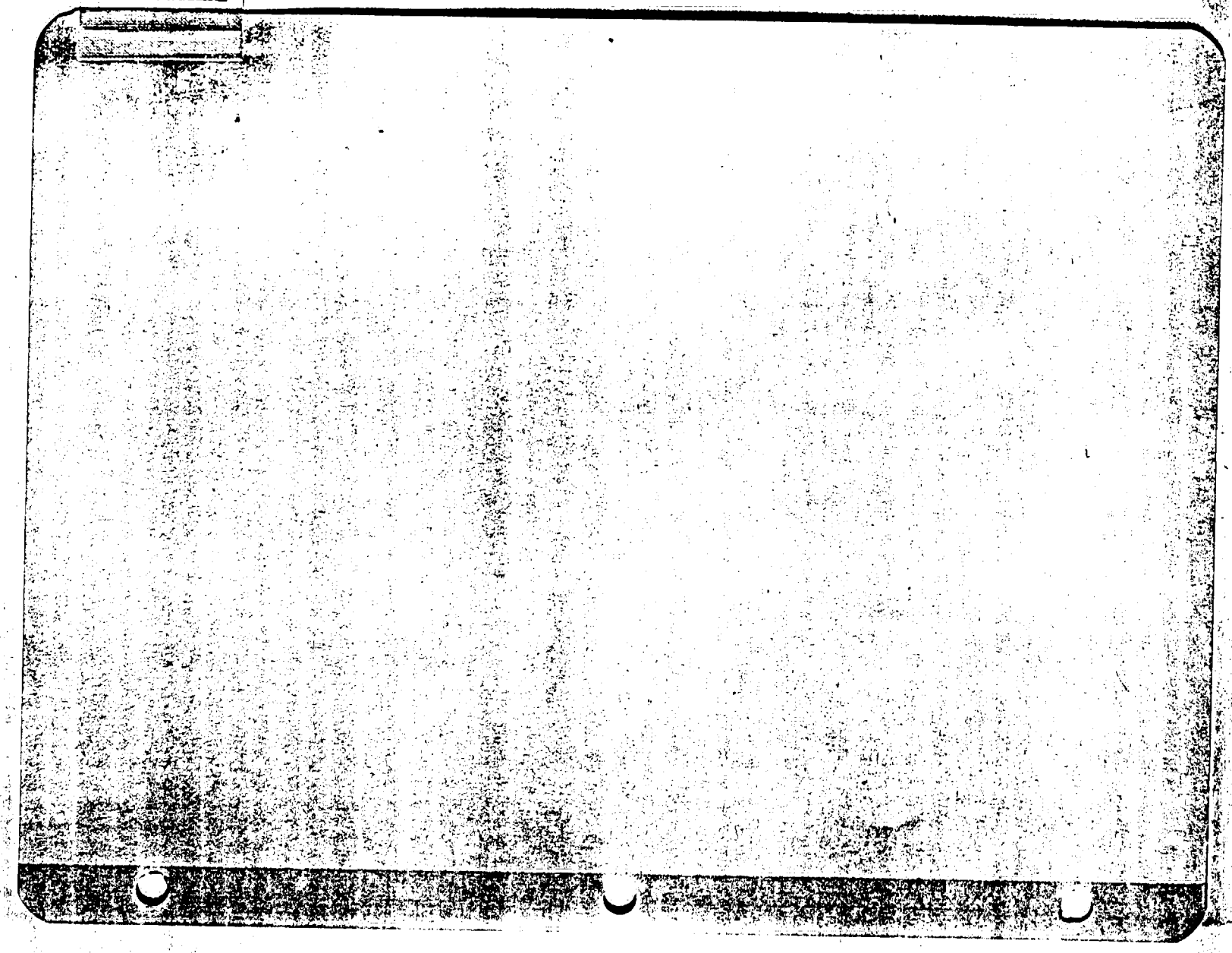

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THE COUNCIL FOR TOBACCO RESEARCH – U.S.A., INC.

February 7, 1973

P-5

Grant Application No. 547CR2

To: The committee comprising Drs. Jacobson, Loosli and Sommers

Subject: Joseph J. Guarneri, Ph.D., Long Island Jewish-Hillside Medical Center, New Hyde Park, N. Y.
Renewal application No. 547CR2
"The Influence of Extended Exposure to Cigarette Smoke on Pulmonary Resistance to Infection as Related to Alveolar Macrophage and Mucociliary Function"

History

CTR support of this investigator goes back to 1960. The current grant is for $20,633.

The enclosed application requests $22,944 for the third and final year of a three year program.

Documents Submitted (attached)

1. Application dated 1/23/73, including a biography and bibliography of Dr. Sierra.

2. Progress report July 1, 1972 - December 31, 1972.

Comment

Dr. Guarneri presented lucidly on "The Influence of Cigarette Smoke on the Alveolar Macrophage Numbers, Viability and Antibacterial Activity" at our Macrophage Conference, November 30 and December 1, 1972.

F.W.N.

FWN:wg
Encls.

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THE LONG ISLAND JEWISH HOSPITAL — QUEENS HOSPITAL CENTER AFFILIATION
82-68 164 ST., JAMAICA, N. Y. 11432 • 212 JA 6-8600

January 23, 1973

Robert C. Hockett, Ph.D.
Acting Scientific Director
The Council for Tobacco Research, U.S.A.
110 East 59th Street
New York, N. Y. 10022

Dear Dr. Hockett: Re: CTR Grant 547C

On January 12, 1973, I was notified by the office of the Council for Tobacco Research, that the application for renewal of our research grant entitled "The Influence of Extended Exposure to Cigarette Smoke on Pulmonary Resistance to Infection as Related to Alveolar Macrophage and Mucociliary Function" was due by January 31, 1973.

The above grant was funded starting July 1, 1971. In the meantime, I accepted a new position effective February 1, 1972, at the present institution. The first year of support was terminated on January 31, 1972 and CTR approval of the grant transfer was received on February 22, 1972. A renewal application and progress report was submitted on March 28, 1972, and the second year of support began on July 1, 1972 at Long Island Jewish-Hillside Medical Center/Queens Hospital Center Affiliation. The present grant request is, as indicated, for renewal beginning July 1, 1973.

Progress reports have been forwarded to the CTR for each year of past support and detailed reports have been submitted for CTR Grants 547B and 547-BR1, covering the periods of investigative work from 7/1/69 to 12/31/69 and 1/1/70 to 12/31/70, respectively. The last detailed progress report was submitted on March 28, 1972 for CTR Grant 547 and covered the grant from 1971 to 1972. The present progress report, No. 7, covers the research period from 7/1/72 to 12/31/72.

In keeping with the recent changes in the renewal dates for CTR grants and the recent relocation of my research laboratories, the present progress report, No. 7, will contain in summary statement of pertinent data present in full detail in progress report No. 6 and this will be related to a status report of my current research efforts from July 1972 to the present.

Thank you for your past considerations, I remain,

Sincerely,

Joseph J. Guarneri, Ph.D.
Attending Microbiologist and
Director of Clinical Microbiology
Laboratories

JJG/pb

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Comm.
Dr. Jacobson
Dr. Loosli
Dr. Sommers

#547CR2
#547CR1-7/1/72-6/30/73
#547C -7/1/71-6/30/72
#547BR -7/1/70-6/30/71
#547B -7/1/69-6/30/70
#547A -7/1/68-6/30/69
#547R -7/1/67-6/30/68
#547 -7/1/66-6/30/67
CF265M - 11/15/60 - 11/15/64 incl.

CHRONIC PULMONARY DISEASES

THE COUNCIL FOR TOBACCO RESEARCH—U.S.A., INC.
110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8565

Application For Renewal of Research Grant
(Use extra pages as needed)

First Renewal ☐ Second Renewal ☐

Date: 1/23/73

JAN 2 9 1973

JAN 2 9 1973

1. Principal Investigator (give title and degrees):
Joseph J. Guarneri, Ph.D., Attending Microbiologist, Director Microbiology Labs, Long Island Jewish-Hillside Medical Center/Queens Hospital Center Affiliation.

2. Institution & address:
Long Island Jewish-Hillside Medical Center
New Hyde Park, N. Y. 11040

3. Department(s) where research will be done ~~or collaboration provided~~
Department of Laboratories
Queens Hospital Center Affiliation, Long Island Jewish Hospital Center
82-68 164th St., Jamaica, N. Y. 11432

4. Short title of study.
The Influence of Extended Exposure to Cigarette Smoke on Pulmonary Resistance to Infection as Related to Alveolar Macrophage and Mucociliary Function.

5. Proposed renewal date. July 1, 1973

6. How results to date have changed earlier specific research aims: None

7. How results to date have changed earlier working hypothesis: None

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8. Any additional facilities now required? Describe briefly: None

9. Any changes in personnel? Append biographical sketches of new key professional personnel: See Page 2A

(a). No change in personnel.

(b) See page 2A for biographical sketch of new professional personnel, Marcelino F. Sierra, Ph.D.

10. Append outline of experimental protocol for ensuing year. See pages 2B - 2D

11. List publications or papers in press resulting from this or closely related work. (append reprints or manuscripts not previously sent).

J. J. Guarneri and G. A. Laurenzi. Influence of Cigarette Smoke on Pulmonary Defense Against Inhaled Bacteria: Alveolar Macrophage Numbers and Viability. ASM Abstracts page 108, 1972.

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12. Summary progress report (append in standard form as separate document, unless recently submitted). Progress Report appended.

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R: REDACTED MATERIAL

Name	Title	Birthdate
Marcelino F. Sierra, Ph.D.	Asst. Attending Clinical Microbiologist	REDACTED

Place of Birth

REDACTED

Sex

Male

Education

Institution	Degree	Year Conferred
City College of New York	B.S. (Biology)	
Wagner College, New York	M.S. (Bacteriology)	REDACTED
St. John's University, New York	Ph.D. (Microbiology-Biochemistry)	

Major Research Interest

Energy metabolism of cells in the normal situation and during impaired function.

Publications

1. Sierra, M.F. and Tzagoloff, A. Properties of a subunit of yeast ATPase synthesized by mitochondrion. Fed. Proc. 31:463 Abstract 1972.

2. Tzagoloff, A., Akai, A., and Sierra, M. Assembly of mitochondrial membrane system VII. Synthesis and integration of F-1 subunits into the rutamycin-sensitive ATPase. Accepted for publication: J. Biol. Chem. (1973)

3. Tzagoloff, A., Meryl, R., and Sierra, M. Biosynthesis of mitochondrial enzymes. Accepted for publication: Biochem. Biophys. Acta. (1973)

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Major Work Planned for Grant Period 7/1/73 to 6/30/74*

Investigative work during the ensuing grant year will include studies of the glucose metabolism and hydrolytic enzyme activity of basal and mobilized alveolar macrophages harvested from control and smoke-exposed animals under the same conditions of smoke-exposure used to assess phagocytosis and intracellular killing activity.** This information is desirable, since energy output and cell metabolism represent important potential links in the successful mobilization and subsequent antibacterial activity of alveolar macrophages. Indeed, alterations in hydrolytic enzyme activity may interfere with the primary immunological function of macrophages in pulmonary defense, namely, the destruction of inhaled microorganisms.

(1) Respiration studies: Experiments are planned which will permit determinations of the oxygen consumption and lactic acid content of alveolar macrophages incubated in basal medium and media supplemented with glucose plus serum with and without the presence of particles that induce phagocytosis. These studies will be performed with the following categories of macrophages: (a) basal macrophages harvested from control and smoke-exposed mice and (b) mobilized macrophages harvested from control and smoke-exposed mice immediately after exposure to aerosols of a phosphate buffer for 30 minutes.

Measurements of oxygen uptake will be determined in a Gilson respirometer using flasks with a 15 ml capacity containing monolayers of alveolar macrophages in a total liquid volume of 3.2 ml(1). The CO_2 will be absorbed by 0.2 ml of 20% KOH in the center well. In accordance with the protocol of each study, glucose (5.0 to 10 M) and polystrene spheres at a concentration of 2.0 to 2.5 mg/ml will be introduced via the side arm. After completion of the oxygen consumption measurements, the cells will be harvested and their lactic acid content determined by the method of Barker et al (2). For this purpose, the recovered macrophages will be washed in saline and cell extracts are to be prepared as described by Myrvik et al (3).

(2) Glucose metabolism studies: Experiments with specifically labelled glucose as substrate will be performed to evaluate the effect of cigarette smoke on the metabolism of glucose by alveolar macrophages. These studies will be done with the same categories of basal and mobilized macrophages used in the respiration studies.

The radioactive measurements will be done as described by Myrvik et al (3). Alveolar macrophages are harvested and placed in Erlenmeyer flasks containing medium 199 without serum or glucose. In separate experiments BCG and heat killed staphylococci will be added to all flasks except the control flasks. After incubation at 37°C in a shaker bath for 1, 2, 4, 6, and 18 hours, glucose 1-C^{14} or glucose 6-C^{14} is added to the flasks, and reincubated for 1 hour. The reaction is stopped with sulfuric acid and counts obtained with a liquid scintilation counter.

(3) Hydrolytic enzyme studies: The present grant proposal will include a study of the enzymatic activity and intracellular distribution of a specific group of hydrolytic enzymes in basal and mobilized macrophages harvested from control and smoke-exposed animals. The enzymes acid phosphatase, lysozyme, lipase, beta-glucuronidase and cathepsin are of immediate interest because their activity is increased in the BCG induced alveolar macrophage (3-6). To accomplish these aims, the enzymatic activity detectable in the cell free supernatant fraction and alveolar macrophage fraction of lung harvests will be assayed. Interest in both fractions of lung harvests resides in the fact that bronchial mucus contains several poorly defined substances(7)

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*The methods, procedures and conditions of smoke and aerosol exposure have been presented in detail in the original proposed project research plan of CTR Grant 547C (Jan. 18, 1971).

**Details of in vitro phagocytosis system has been presented in detail in Progress Report No. 1 for Grant #547-B covering the grant period 7/1/69 - 12/31/69.

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including lysozyme (8) that exert nonspecific bacteriostatic and bactericidal activity against gram positive and gram negative bacteria. In addition, alveolar macrophages by virtue of their high hydrolytic enzyme content, presence in large numbers and rapid turnover rate may contribute to the enzymatic activity found in mucus secretions.

3(a) Enzyme activity of lung harvests: A comparison will be made of the enzymatic activity and intracellular distribution of the above hydrolases in the following categories of macrophages: (a) basal macrophages harvested from control and smoke-exposed animals and (b) mobilized macrophages harvested from control and smoke-exposed animals immediately after exposure to aerosols of viable or heat-killed staphylococci. For this purpose, smoke-exposed and control animals will be exposed to the bacterial aerosols for 30 minutes. Enzyme determinations will be made with the lung harvests obtained from both groups of animals immediately after bacterial challenge and at hourly intervals over a 4 hour post-aerosol exposure period. The choice of laboratory animals (mice, guinea pigs, rabbits) to be used in all enzyme studies will be governed by the minimal yields necessary for adequate enzyme assay. The lung washing obtained from animals by the lavage technique will be separated into a cell-free supernatant fraction and cellular fraction containing alveolar macrophages by centrifugation at 4°C for 15 min at 10,000 rpm (8). The supernatant will be frozen at -60°C and stored until assay. The harvested alveolar macrophages will be washed twice in phosphate buffered saline (pH 7.2); quantitated by hemocytometer count; and viability assessed. Saline extracts of the washed cells are to be prepared for enzyme activity studies as outlined by Myrvik et al(3): disruption by alternate freezing and thawing for 5 consecutive cycles and removal of cellular debris by centrifugation at 2,500 rpm for 10 min at 4°C. The completeness of cell disruption will be audited by phase optics. In order to evaluate the contribution made by alveolar macrophages to the enzymatic activity detectable in the supernatant fraction, in vitro studies of the rate of release of the enzymes in question by pulmonary macrophages are planned. As outlined by Holzman et al (8) large numbers of alveolar macrophages will be suspended in tissue culture medium and samples of the cell population assayed for specific enzymatic activity after incubation at 37°C for various periods of time. In studies of the intracellular distribution of the hydrolases, alveolar macrophages will be suspended in a 0.25M sucrose solution, ruptured by homogenization and sedimented by centrifugation into four fractions (4). The nuclear fraction (N) will be sedimented by centrifugation at 250 X G; the heavy granule fraction (HG) at 5000 X G for 15 min; and the light granular fraction (LG) and supernatant fraction (S) by centrifugation at 25,000 X G. Each fraction will be submitted to 5 cycles of freezing and thawing and clarified by centrifugation at 2500 X G for 20 min and subjected to enzyme assay.

3(b) Antibacterial activity of supernatant fraction of lung harvests: In order to study the antibacterial properties of pulmonary secretions, the supernatant fraction of lung harvests obtained by the lavage technique will be inoculated with fixed numbers of viable bacteria, incubated at 37°C for various periods of time and the viability of the bacteria assessed by colony counts obtained by a standard agar pour plate technique. The microorganisms to be used as a bacterial challenge include S.aureus, S.albus, other gram positive bacteria and several gram negative bacteria. These studies will be performed with the supernatant fraction of lung harvests obtained from the following categories of animals: (a) basal macrophages harvested from control and smoke-exposed animals and (b) mobilized macrophages harvested from control and smoke-exposed animals challenged with aerosols of carbon particles or heat-killed staphylococci.

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For the purpose of enzyme analysis, lysozyme will be quantitated by using suspensions of Micrococcus leisodyktious as substrate. Tests will be standardized with known amounts of crystalline egg white lysozyme and results expressed as egg white lysozyme equivalents. Acid phosphatase will be measured by the procedure of Hofstee (9) using O-carboxyphenyl phosphate as substrate. An increase in absorbance of 0.001 optical density units/min under standard conditions will be taken to represent a unit of activity. Beta-glucuronidase will be assayed by the procedure of Fishman et al (10) using phenolphthalein mono-Betaglucuronide as substrate with reactions carried out in 0.1M acetate buffer at pH 4.5 and 38°C. One unit of glucuronidase is the activity resulting in the liberation of 1 ug/hr phenolphtalein. Lipase activity will be determined as outlined by Cohn et al (5) in which naphthol laurate serves as substrate and the increase in activity caused by sodium taurocholate is taken to represent minimal lipase activity. The results are to be expressed as micromoles of naphthol liberated per hour. Cathepsin will be assayed employing a 2% solution of denatured hemoglobin as a substrate, as described by Anson (11) and protein digestion estimated with a spectrophotometer by absorption at 280 mu (4). A unit of activity being defined as an increase in optical density produced by 0.001 meq of tyrosine.

References

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2. Barker, S. B. and Summerson, W. H.: The colorimetric determination of lactic acid in biological material, J. Biol. Chem. 138: 535, 1941.

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4. Meise, E. R., Myrvik, Q. N. and Leake, E. S.: Effects of Bacillus Calmette Guerin on the levels of acid phosphatase, lysozyme and cathepsin in rabbit alveolar macrophages, J. Immun. 95: 125, 1965.

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8. Holzman, R. A., Gardner, D. E. and Coffin, D. L.: In vivo inactivation of lysozyme by ozone, J. Bacterial 96: 1562, 1968.

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13. Budget for the coming year:

	hrs/week % time	Amount
A. Salaries (give names or state "to be recruited") Professional (give % time of investigator(s) even if no salary requested) (See Page 3A)		
Joseph J. Guarneri, Ph.D. Principal Investigator	20 hrs/week	REDACTED
Marcelino Sierra, Ph.D.	10 hrs/week	
Technical		
Michael Veit, M.S.	100%- 35 hrs/wk. Fringe benefits	
Research Assistant	30% - 12 hrs/wk. Fringe benefits	
Sub-Total for A		
B. Consumable supplies (by major categories) (See page 3A for itemization)		
Sub-Total for B		$4,650
C. Other expenses (itemize) Travel $350 Publications 150		
Sub-Total for C		$ 500
Running Total of A + B + C		$18,577
D. Permanent equipment (itemize) (see Page 3A) Gilson Differential Respirometer with 8 volumometers and mobile base (Model G 14)		
Sub-Total for D		1,580
E. Indirect costs (15% of A+B+C)		2,787
Total		$22,94[illegible]

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Justification and Explanation of Budget

Budget Category: (A) Salary: The increase over 1972 in the salary category of $972 reflects the anticipated increment in salaries and accompanying fringe benefits during the grant period of 7/1/73 to 6/30/74.

Budget Category: (B) Itemization of Consumable Supplies:

Mice Swiss Webster, Caesarian Delivered, including feed	$ 1,000
Rabbits	1,100
Cigarettes for smoke studies	800
Petri dishes, plastic disposable	200
Tissue culture glassware, media and serum	600
Bacteriology media and defibrinated blood	200
Isotapes, uniformly labelled glucose -1-C^{14} and glucose 6-C^{14}	300
General Lab, glassware and reagents	150
Reagents and curvettesfor enzyme studies and flasks and accessories for respiration studies	300
	$4,650

Budget Category: (D) Permanent Equipment

The Gibson Differential Respirometer is needed for the respiration and isotopes studies proposed in the original grant request and the current grant renewal. The respirometer was not included in the projected equipment needs of the original grant request made in 1971 because it was then available at St.Vincent Hospital. At the present institution this instrumentation is not available to my research laboratory.

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14. Other sources of financial support.

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
(1) The Influence of Extended Exposure to Cigarette Smoke on Pulmonary Resistance to Infection as Related to Alveolar Macrophage and Mucociliary Function.	The Council for Tobacco Research-USA. Grant Nos. 547 and 547C	$40,208	7/1/71 to 6/30/73

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
(1) The Influence of Extended Exposure to Cigarette Smoke on Pulmonary Resistance to Infection as Related to Alveolar Macrophage and Mucociliary Function.	The Council for Tobacco Research-USA. (Current renewal application)	$22,944	7/1/73 to 6/30/74
(2) The Influence of Bacterial Species on the In Vitro Antibacterial Activity of Alveolar Macrophage.	Long Island Jewish-Hillside Medical Center (to be submitted 7/1/73)	6,000	7/1/73 to 6/30/74
(3) The Mechanisms of Pulmonary Defense Against Infection.	Dep't. Health, Education and Welfare, Public Health Services (To be submitted 6/1/73)	180,000	1/1/74 to 12/31/77

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name Joseph J. Guarneri, Ph.D.

Signature [redacted] Date 1/24/73

Telephone [redacted]
Area Code Number Extension

Checks payable to

Long Island Jewish-Hillside Medical Center

Mailing address for check: Mrs. Eva Meyers, Grant Mgr.
Long Island Jewish-Hillside Medical Center
New Hyde Park, New York 11040

Responsible officer of institution

Typed Name Robert K. Match, M.D.

Title Executive Vice President & Director

Signature [redacted] Date

Telephone [redacted]
Area Code Number Extension

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

Joseph J. Guarneri, Ph.D.
Long Island Jewish-Hillside Medical Center
Queens Hospital Center Affiliation
82-68 164th Street
Jamaica, N. Y. 11432

Dates covered:
July 1, 1972 - December 31, 1972

The Influence of Extended Exposure to Cigarette Smoke on Pulmonary Resistance to Infection as Related to Alveolar Macrophage and Mucociliary Function.

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Status Report of Research Covering Grant Period 7/1/72 to 12/31/72	7 - 10

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Joseph J. Guarneri, Ph.D.
Long Island Jewish-Hillside Medical Center
Queens Hospital Center Affiliation
82-68 164th Street
Jamaica, N. Y. 11432

Dates covered:
July 1, 1972 - December 31, 1972

The Influence of Extended Exposure to Cigarette Smoke on Pulmonary Resistance to Infection as Related to Alveolar Macrophage and Mucociliary Function.

Contents of Progress Report No. 7

On May 28, 1971, I received notification from W. T. Hoyt, Executive Vice President of the Council for Tobacco Research-USA, Inc., that the application for our research grant entitled "The Influence of Extended Exposure to Cigarette Smoke on Pulmonary Resistance to Infection as Related to Alveolar Macrophage and Mucociliary Function" was approved for support. The grant was funded starting July 1, 1971. In the meantime, I accepted a new position at the present institution and the first year of support was terminated on January 31, 1972. The move took place in February and the first year of support was continued in March 1972. A renewal application was submitted on March 28, 1972, and the second year of support began on July 1, 1972, at Long Island Jewish-Hillside Medical Center/Queens Hospital Center Affiliation. The present request is, as indicated, for renewal beginning July 1, 1973. Although progress reports have been submitted for each year of past support, the most complete ones are Progress Reports Numbers 4 and 5, covering the periods 1969 to 1970 and 1970 to 1971. The last detailed progress report (No. 6) was submitted on March 28, 1972, covering the grant period of 1971 to 1972. In keeping with the recent changes in the renewal dates for CTR Grants and the recent relocation of my research laboratories, the present progress report (No. 7) will include a summary statement of previous detailed reports and this will be related to a status report of my current research efforts from July 1972 to the present.

1003539336

Summary Statement*
of Progress Reports Nos. 5 and 6

There is ample clinical and experimental evidence which demonstrates that the normal lung tends to maintain a sterile atmosphere; namely, it is free of viable bacteria. Evidence for the antibacterial activity of the broncho pulmonary tissue of human beings and experimental animals, respectively, resides in the observations that bronchial secretions of normal human subjects are sterile and that bacteria deposited in the lungs of animals are rendered non-viable within hours.

Although several factors undoubtedly contribute to the clearance of foreign material deposited in the lungs, the mucociliary apparatus and alveolar macrophage defense systems comprise the major defenses of the respiratory tract. Attention has been focused on the alveolar macrophage system by studies completed in this laboratory, as well as others, which demonstrate that alveolar macrophages are mobilized in response to the inhalation of viable and non-viable bacteria, cigarette smoke and carbon dust. Mobilization provides a mechanism by which large numbers of macrophages are made available to sites of pulmonary insult and therefore may be intimately associated with the processes that permit the lung to maintain a sterile atmosphere.

*The materials, methods and data have been presented in detail in Progress Reports numbers 5 and 6.

Source: https://www.industrydocuments.ucsf.edu/docs/yfyl0000

Joseph J. Guarneri, Ph.D.
Long Island Jewish-Hillside Medical Center
Queens Hospital Center Affiliation
82-68 164th Street
Jamaica, N. Y. 11432

Dates covered:
July 1, 1972 - December 31, 1972

The Influence of Extended Exposure to Cigarette Smoke on Pulmonary Resistance to Infection as Related to Alveolar Macrophage and Mucociliary Function.

The studies reported in Progress Reports Numbers 5 and 6 are concerned with the influence of acute exposure to cigarette smoke on pulmonary resistance to bacterial infection and utilizes methods for investigating the quantitative aspects of pulmonary defense as related to the alveolar macrophage. Specifically, the influence of smoke inhalation was assessed in terms of its effect on the numbers of available macrophages, cell viability and antibacterial activity. In addition, by utilizing an in vitro phagocytosis system and in vivo and in vitro conditions of smoke exposure, it has been possible to evaluate the following aspects of the antibacterial activity of rabbit alveolar macrophages in the normal situation and during exposure to cigarette smoke: (1) the influence of culture conditions and serum factors on antibacterial activity in the normal situation and during exposure to cigarette smoke, (2) the importance of changes in culture media pH induced by cigarette smoke on macrophage function, (3) the role of increased macrophage numbers (mobilization) as a determinant of the in vitro antibacterial capacity of an alveolar macrophage population in the presence and absence of cigarette smoke, (4) the influence of alveolar macrophage activity on the toxicity of a smoke solution (5) the antiphagocytic effect of in vivo smoke exposure on the acellular fraction (pulmonary lavage solution) of lung harvests, (6) the relative effect of whole smoke and the gas phase of whole smoke on the antibacterial properties of macrophages (7) the reversibility of changes in macrophage function induced by exposure to whole cigarette smoke under in vitro and in vivo conditions of smoke exposure and (8) to compare the effects of whole cigarette smoke on the antibacterial activity of macrophages under in vitro and in vivo conditions of smoke exposure.

1003539337

Materials and Methods

A. In Vitro Phagocytosis System: Alveolar macrophages harvested from rabbits were placed in tissue culture flasks containing Hanks' solution supplemented with normal rabbit serum and challenged with S. aureus. At specific intervals after bacterial challenge, the reduction in the number of viable staphylococci present in the extracellular fraction of tissue culture preparations was used to determine the percent bacteria phagocytized while the number of viable bacteria cultured from monolayers (macrophage fraction) provided estimates of the percent intracellular survival of ingested bacteria. Direct studies of the bactericidal capacity of macrophage cultures were also performed in which the ingestion and post-ingestion phases of staphylococcal inactivation were dissociated by washing monolayers after bacterial challenge. A standard pour plate technique was used to obtain bacterial counts from the extracellular and macrophage fractions immediately after bacterial challenge and at various intervals, thereafter. Under these conditions, it is possible to quantitate the following parameters of alveolar macrophage function: (a) the percent bacteria phagocytized by macrophages, (b) the percent phagocytized bacteria surviving within macrophages, (c) the percent bacteria killed by macrophage cultures over a 1.5 hour test period and (d) a direct assessment of bactericidal capacity of macrophages. The methods, procedures and details of the in vitro phagocytosis system have been presented in detail in Progress Report No. 1 for Grant #547-B.

Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

Joseph J. Guarneri, Ph.D.
Long Island Jewish-Hillside Medical Center
Queens Hospital Center Affiliation
82-68 164th Street
Jamaica, N. Y. 11432

Dates covered:
July 1, 1972 - December 31, 1972

The Influence of Extended Exposure to Cigarette Smoke on Pulmonary Resistance to Infection as Related to Alveolar Macrophage and Mucociliary Function.

B. Conditions of In Vitro Smoke-Exposure: A commercial brand of non-filtered cigarette was attached to a 30 ml. syringe by a rubber tube and smoke was produced by withdrawing the barrel of the syringe at a rate of approximately 18 ml. per second. Six successive "puffs" of cigarette smoke were introduced into the syringe and emptied by removing a rubber tube containing the lighted cigarette. The smoke from the seventh puff was introduced into the tissue culture flask with a sterile hypodermic needle. The protocol of each study included control flasks containing only bacteria and alveolar macrophages challenged with bacteria respectively. Under these conditions, both categories of tissue culture preparations were exposed for 1.5 hours to whole cigarette smoke and whole smoke drawn through a glass fiber filter.**

C. Conditions of In Vivo Smoke-Exposure: A smoke generating apparatus*** was used to deliver puffed cigarette smoke under controlled conditions. In the studies reported herein, the apparatus was adjusted to deliver a 35 ml. puff of 2 seconds duration from a commercial brand of non-filtered cigarettes. The time interval between puffs from a single cigarette was set at 58 seconds. The instrument is equipped with a rotating disc which can accommodate 30 cigarettes at one time, so that it is possible to maintain a continuous stream of puffed smoke generated at a rate of 1 L/min. The initial smoke delivered by the cigarettes is diluted and transported across an animal exposure chamber by a secondary air flow. Control animals are placed in a plexiglass chamber and exposed to a secondary air flow. In the present experiments, smoke-exposed and control animals were exposed to whole cigarette smoke and a secondary air flow, respectively, for 1.5 hours.

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Discussion of Results and Significance of the Data

In the reported studies, smoke inhalation proved an effective means of inducing a macrophage response. Specifically, smoke inhalation provoked an increase in the numbers of alveolar macrophages available to the lung without any adverse effect on cell viability. In addition, it did not stimulate the migration or proliferation (mobilization) of other phagocytic cells such as polymorphonuclear leukocytes. These observations demonstrate that one of the immediate effects of smoke inhalation on the cellular defense mechanisms of the respiratory tract is a direct stimulation of the alveolar macrophage defense system. The data further suggests that the presence of increased macrophages may play a central role in protecting the lung against smoke toxicity. To postulate such a protective role for these cells is supported by studies performed under in vitro conditions of smoke exposure and presented in Progress Report No. 6 which demonstrate the following: (1) increased numbers of macrophages reduce or limit the adverse effect of cigarette smoke on the antibacterial activity of a macrophage population in vitro and (2) alveolar macrophages are capable of inactivating toxic components present in a smoke solution that impair the antibacterial activity of normal alveolar

**Cambridge glass fiber filter - Phipps and Bud, Richmond, Va.
***The details and calibration data of the smoke-exposure system has been presented in detail in Progress Report No. 6.

Source: https://www.industrydocuments.ucsf.edu/docs/[illegible]

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Dates covered:
July 1, 1972 - December 31, 1972

The Influence of Extended Exposure to Cigarette Smoke on Pulmonary Resistance to Infection as Related to Alveolar Macrophage and Mucociliary Function.

macrophages in vitro.

The data obtained from a direct investigation of the phagocytic and bactericidal properties of alveolar macrophages harvested from smoke-exposed animals (in vivo conditions of smoke-exposure) has provided additional pertinent information. As demonstrated by the pulmonary lavage studies, exposure to cigarette smoke caused an accumulation of "toxic substances" that can be washed out of the lungs and inhibit the antibacterial activity of alveolar macrophages in vitro. However, smoke inhalation did not permanently impair the antibacterial activity of the alveolar defense system. Specifically, smoke-exposure caused an impairment of the phagocytic and bactericidal powers of macrophage harvests that was readily reversible within hours. The fact that macrophage function was restored under in vitro conditions of recovery; namely, by multiple washing and incubation of macrophage in a smoke-free environment demonstrates the capacity of the alveolar macrophage system to cope with cigarette smoke and the importance of in vivo processes which effectively remove inhaled products of cigarette smoke from the respiratory tract. To correlate with this, the adverse effect of smoke inhalation was reversed under in vivo conditions of recovery, namely, by housing smoke-exposed animals in a smoke-free room prior to obtaining lung harvests. Collectively, these observations suggest that the respiratory tract responds to the presence of cigarette smoke, as well as other foreign materials in a similar manner; namely, pulmonary defense mechanisms are activated and respond by removing and/or inactivating smoke products deposited in the lung. It appears reasonable to assume that the efficacy of this response is facilitated by the in vivo mobilization of increased macrophage numbers in relation to the inhalation of cigarette smoke. Such an interpretation of the data would be in keeping with experimental evidence previously cited in this report which demonstrates that alveolar macrophages may play a key role in protecting the lung against the toxic effects of cigarette smoke.

Studies performed under in vitro conditions of smoke-exposure have served to provide: (1) the laboratory techniques necessary for more meaningful studies of the effect of cigarette smoke on the living and functioning lung and (2) a better understanding of the conditions of bacterial challenge and smoke-exposure that influence the antibacterial properties of alveolar macrophages. As demonstrated by studies presented in Progress Reports Numbers 5 and 6, the antibacterial capacity of a macrophage population in the absence and presence of cigarette smoke is enhanced by the presence of increased numbers of alveolar macrophage. This observation and the detoxification data presented earlier, support published reports which have focussed attention on the importance of alveolar macrophage mobilization as a pulmonary defense mechanism against inhaled infectious and toxic agents. Results of other studies performed under in vitro conditions of smoke-exposure indicate that the local conditions necessary for effective antibacterial activity by normal and smoke-exposed macrophages differ. In this regard, the antibacterial activity of macrophages during smoke-exposure was significantly influenced by the concentration of serum in the culture medium. This effect could not be accounted for by the presence or absence of an added carbon source (glucose) in the culture medium or the increased acidity of the culture medium induced by cigarette smoke. The fact that exposure to cigarette smoke interfered with the antibacterial activity of alveolar macrophages only at serum concentrations of 2.5% or less indicates that serum factors may pro-

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tect against smoke toxicity. This may be related to specific immunological and/or nutrient components of serum. In addition, it has also been observed that the protective action of serum is reduced if cigarette smoke is introduced prior to bacterial challenge. Specifically, prior exposure of macrophage cultures to cigarette smoke reduced the amount of cigarette smoke necessary to suppress antibacterial activity. This difference may be a reflection of the long term effects of cigarette smoke on a static macrophage population and probably relates to several factors including the effect of extended exposure to cigarette smoke on: (1) serum factors which promote and sustain effective macrophage function and (2) the physiological state of macrophages at the time of bacterial challenge. Macrophages obviously respond to the presence of cigarette smoke by removing and/or detoxifying smoke products in their environment. The fact that the adverse effects of in vitro smoke-exposure was partially reversed by multiple washing of macrophage cultures demonstrates the beneficial results derived from reducing the amount of smoke in contact with the alveolar cells and the importance of the cleansing action of increased numbers of macrophages (mobilization) and mucociliary activity in the live and functioning lung. Data has also been presented which clearly demonstrates that exposure to whole smoke drawn through a glass fiber filter reduced the adverse effect of in vitro smoke-exposure on the antibacterial activity of macrophages. Specifically, filtered whole smoke did not interfere with phagocytic activity but continued to impair bacterial killing. These observations suggest that the particulate and gas phases of whole smoke may have separate effects on macrophage function.

Marked differences in the effect of in vivo and in vitro smoke-exposure on macrophage function have also been noted. In vitro conditions of smoke-exposure produced an impairment of antibacterial activity that was not totally reversed by multiple washing and incubation of macrophages in smoke-free tissue culture flasks. The differences in the effects of in vivo and in vitro smoke-exposure on macrophage function may be attributable to several factors including: (1) differences in the amount of smoke that macrophages were exposed to under both experimental conditions and (2) the static and unphysiological conditions that prevail under in vitro conditions of smoke-exposure. The latter interpretation of the data would be in keeping with the mobilization and detoxification data reported herein. Since it is apparent that in vitro findings may not parallel the in vivo process, studies will be performed to determine the influence of acute and extended exposure to whole and filtered smoke on pulmonary defense as related to alveolar macrophage function under in vivo conditions of smoke-exposure.

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The Influence of Extended Exposure to Cigarette Smoke on Pulmonary Resistance to Infection as Related to Alveolar Macrophage and Mucociliary Function.

Status Report of Research Covering Grant Period 7/1/72 to 12/31/72

In keeping with the experimental design outlined in the original grant request studies are in progress to evaluate the influence of extended exposure to cigarette smoke of pulmonary resistance against inhaled bacteria. The latter studies involve housing mice in our laboratory for long periods of time during exposure to cigarette smoke and prior to bacterial aerosol studies. For these reasons it was necessary to determine the influence of the laboratory environment on the sterility of the broncho-pulmonary tree of normal mice and to standardize all procedures necessary to perform meaningful bacterial aerosol and clearance studies. In addition, if animal age is a significant factor, it would be essential to control age in any experiment designed to assess the effect of cigarette smoke on pulmonary defense against inhaled bacteria.

Materials and Methods****

Animals: White male Swiss Webster mice (Charles River - CR-1 strain) were used in all experiments. The animals were housed in plastic cages, 4 per cage, and were permitted access to food and water ad libitum. The lungs of this strain of mice are normally free of detectable viable bacteria. To avoid exogenous bacterial contamination, the animals are shipped to the laboratory in filtered crates.

Aerosol Challenge: A coagulase positive strain of Staphylococcus aureus (FDA 209, phage type 42D) is prepared for aerosolization in the following manner. The second of two successive 12 hour trypticase soy broth cultures are harvested by centrifugation at 6,000 rpm for 20 minutes. The supernatant fraction is discarded and the remaining bacterial pellet is washed with a phosphate buffer (11.36g Na_2HPO_4 and 2.72g KH_2PO_4 per liter) at pH 7.4. Desired concentrations are achieved by resuspending the pellet in appropriate volumes of the phosphate buffer. The number of viable staphylococci present in bacterial suspensions is determined from nutrient agar pour plates incubated at 37° C for 48 hours.

The bacterial aerosol exposure apparatus includes: (a) an aerosol-generating system, (b) a mixing chamber, (c) an animal exposure chamber and (d) a filter-discard system. Aerosols of staphylococci are generated from a buffered suspension of bacteria contained in eight DeVilbiss #40 glass nebulizers. The initial spray from each nebulizer is directed into a mixing chamber with an interposed baffle to remove large droplets and mixed with a secondary airflow of 100 cubic feet per minute which serves to dilute, dry

****The methods of aerosol formation and bacterial aerosol exposure system have been previously described. G.A. Laurenzi and J.J. Guarneri, 1966, Amer. Rev. Respiratory Diseases Vol. 93: 134-141.

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Source: https://www.industrydocuments.ucsf.edu/docs/ygxf0000

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and direct the bacterial aerosol through a large animal exposure chamber. The volume of test bacterial solution nebulized is recorded and the concentration of viable airborne bacteria in the animal exposure chamber is determined from pour plates made of air samples collected with glass impinger samplers containing 20 ml of the phosphate buffer. Particle size determinations are made with an Anderson sampler modified by the manufacturer to separate the aerosol into 8 particle size ranges. A sample of exposure chamber air is drawn through the sampler and the number of viable staphylococci collected in each stage is determined by direct colony count or with the aid of a microscope after a short period of incubation at 37° C.

Bacterial Clearance Studies: Studies of lung clearance are performed in which mice are exposed for 30 minutes to aerosols of phosphate-buffer suspensions of viable staphylococci. Immediately after exposure (0 time) one group of mice is killed and the remainder are sacrificed at various intervals post-aerosol exposure. The lungs are removed aseptically; individually ground in glass homogenizers containing nutrient broth; and bacterial counts obtained from nutrient agar pour plates incubated for 48 hours at 37°C. Under these circumstances, it is possible to determine the number of viable bacteria present in the lungs at 0 time (bacterial deposition) and at each designated interval during the post-aerosol exposure period. From these data lung clearance rates are calculated by expressing the number of bacteria remaining in the lung at each interval after aerosol exposure as a percent of the number deposited at 0 time and subtracting this value from 100%. The term clearance as used in these studies refers to the numbers of bacteria whose presence in a culturable state can no longer be demonstrated.

Results

A. The Influence of the Laboratory Environment on the Sterility of the Respiratory Tract of Test Mice: For this purpose mice were housed in the laboratory for 4 weeks and different groups of mice sacrificed immediately upon delivery to the laboratory and at weekly intervals thereafter. The presence or absence of bacteria was determined from pour plates made of lung homogenates.

As previously stated, the lungs of this strain of Charles River Mice are normally free of viable bacteria. The data presented in Table I demonstrates that conditions of housing and laboratory environment did not cause pulmonary colonization of bacteria. This is indeed remarkable since ambient conditions were provided and therefore the inhalation of airborne bacteria present in the environment and the aspiration of bacteria normally present in the oral-pharyngeal regions is an inevitable occurrence. The data also emphasizes the remarkable capacity of the lower respiratory tract to maintain a sterile atmosphere and the efficacy of the antibacterial mechanisms available to normal lungs.

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Conclusions: From the data obtained to date it is also reasonable to assume that the conditions of animal quarters established at Queens Hospital Center are adequate and provide appropriate controls for studies of the influence of extended exposure to cigarette smoke on the normal defense mechanisms of the pulmonary system.

B. Aerosol Studies - The Particle Size Distribution of the Bacterial Aerosols Used to Deposit Staphylococci in the Lungs of Test Mice: Particle size is a major determinant of the probable site of deposition of inhaled airborne particles in the respiratory tract. The importance of particle size resides in the fact that particles which are deposited on the bronchial tree are subjected to the cleansing action of the mucociliary apparatus and those which deposit in alveoli are phagocytized by alveolar macrophages. Therefore, the mode of clearance is dependent upon the site of deposition, which is in turn a function of particle size. It is also apparent that particle data is mandatory for standardization of test conditions from one experiment to the next and the interpretation of data from multiple studies performed over a long period of time.

Particle size determinations were made 15 minutes after aerosolization of phosphate-buffered suspensions of staphylococci. The cummulative percent of viable bacteria collected on each impaction stage of the Andersen sampler was plotted against particle diameter on logarithmic probability paper using the 50% cut-off of that stage. A line of best fit was drawn by eye and the count median diameter (CMD) of the aerosol was derived by reading the size corresponding to the 50% intercept on the graph. As shown in Figure 1, the data obtained from a single experiment indicates that the bacterial aerosol had a CMD of 1.9 μ and a geometric standard deviation of 1.6 μ. In five separate aerosol studies the average CMD, and cumulative percent particles $\leq$ 3.3 μ, was 1.9 μ and 86%, respectively, through exposure chamber concentrations of 3.6×10^3 to 1.1×10^7 staphylococci per ft^3 of air (Table II).

Conclusions: The data demonstrates that the aerosol system produces bacterial aerosols of controlled concentrations and particle size from which predictable numbers of staphylococci can be implanted in the lungs of mice.

C. Bacterial Clearance Studies: Lung clearance studies were performed to provide the following information: (1) the influence of the laboratory environment on the deposition and clearance of large numbers of staphylococci from the lungs of mice and (2) the relationships which may exist between animal age, body weight and the number of bacteria deposited by aerosol exposure, and clearance rates. This information is necessary to plan and interpret the results of studies in which animals will be exposed to cigarette smoke over a 4 or 5 week period prior to bacterial aerosol challenge.

In some studies groups of mice were challenged with staphylococcal aerosols for 30 minutes 1 day after arriving at the laboratory, and in other experiments mice were exposed to bacterial aerosols 1, 2, 3 and 4 weeks after receipt. Bacterial deposition and 4 hour clearance rates were determined as outlined under Materials and Methods. In separate studies, animal weights recorded prior to bacterial aerosol exposure were correlated

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with mean deposition values. The results of a study in which animal weights and mean deposition values were recorded are presented in Table III. As shown in Table III, exposure of mice ranging in weight from 24.2 g to 50.0 g to the same concentration of airborne Staphylococci resulted in a weight related increase in the number of bacteria deposited in the lungs. Bacterial deposition values of 70,000, 120,000 and 168,000 were achieved in mice weighing 24.2g, 37.2g and 50.0g, respectively.

The data covering the influence of the laboratory environment and animal age on bacterial clearance is presented in Table IV. Bacterial clearance by animals housed in the laboratory from 1 day to 4 weeks was essentially the same.

Conclusions: The data demonstrates the importance of monitoring body weight and environmental conditions during long term studies and suggests that the laboratory facilities at Queens Hospital are adequate for the experiments proposed in the original grant request.

Comments

The methods described herein offer a predictable quantitative technique for studying pulmonary resistance to airborne infection. The method includes: (1) the production of bacterial aerosols of controlled concentration and particle size, (2) the deposition of predictable numbers of organisms in the lungs of test animals (3) the demonstration of rapid clearance of bacteria in the normal situation and (4) the measurement (assay) of significant changes in clearance produced by experimental conditions. The high degree of predictability offered by the aerosol system makes it especially adaptable as a reference challenge-response system to study the influence of extended exposure to cigarette smoke on pulmonary resistance to infection. In this regard, the feasibility of the proposed long term studies has been established by the data obtained to date which indicates that the laboratory environment and animal age do not compromise the pulmonary defense mechanisms of the test animals in our experimental model.

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Source: https://www.industrydocuments.ucsf.edu/docs/ytvl0000

Table 1. The Effect of Exposure to Laboratory Conditions on the Sterility of the Murine Lung.

No. of Animals	No. of Days Housed in Laboratory	No. of Animals from which Bacteria* were isolated from their lungs.
30	1	1
30	7	1
30	14	0
30	21	1
30	28	2

*The number of organisms isolated ranged from 1 to 10 organisms/lung.

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Source: https://www.industrydocuments.ucsf.edu/docs/vtyl0000

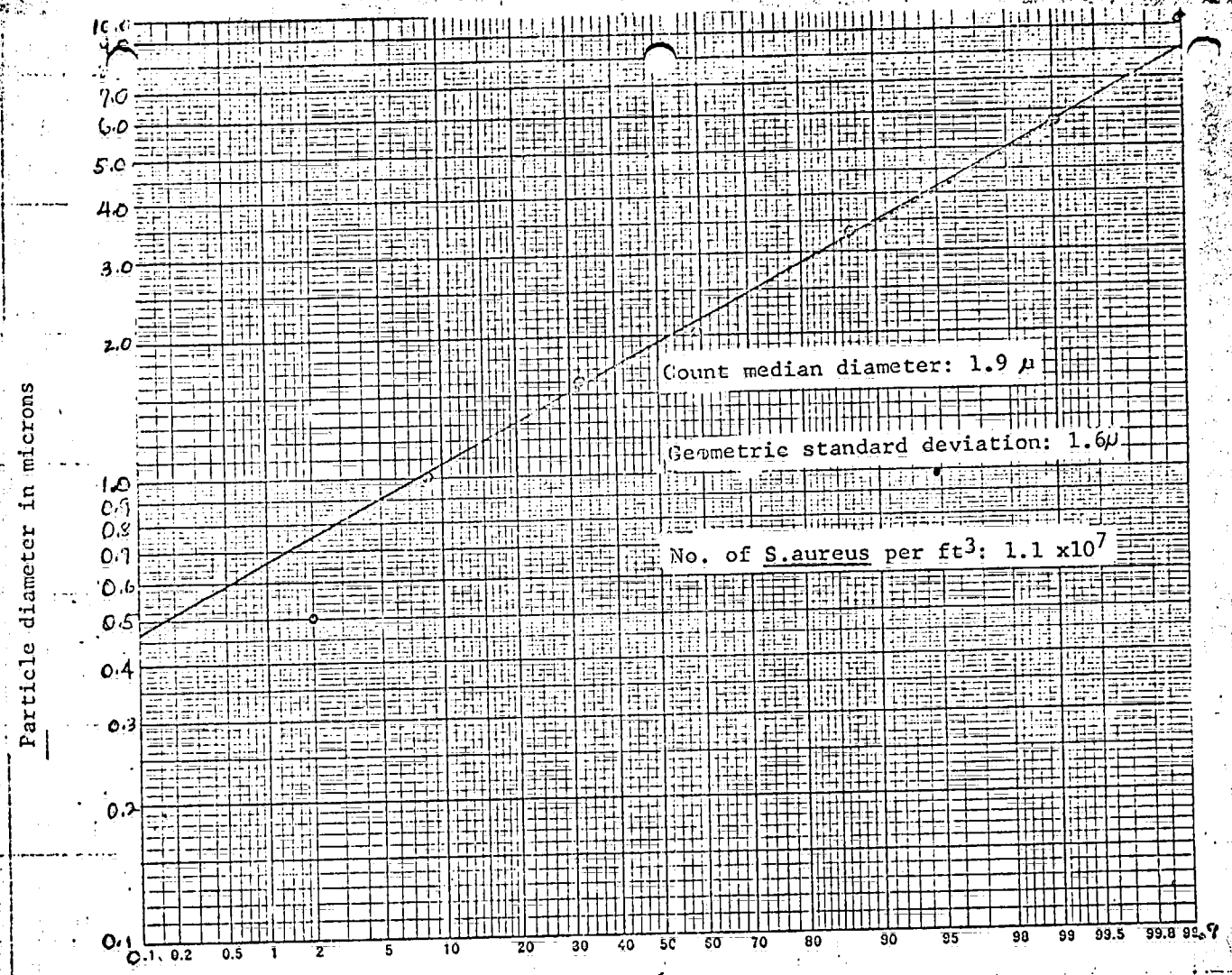

Fig.1 Particle size distribution of a staphylocccal aerosol.

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

Table II Particle size distribution of the staphylococcal aerosols.

No. of S.aureus per ft^3 of air	Count Median Diameter μ	Geometric Standard Deviation μ	Cumulative % $\leq$ 3.3 μ
3.6×10^3	1.9	1.7	87
2.3×10^4	1.8	1.7	87
2.2×10^5	2.1	1.6	84
1.0×10^6	1.9	1.8	84
1.1×10^7	1.9	1.6	87

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

Table III. The Numbers of Staphylococci Deposited in the Lungs of Mice of Various Weights Exposed to S. aureus at a Chamber Concentration of 2.1×10^6 organism/ft^3.

Mouse Weight (gms)	Number of S. aureus Deposited in the Lungs ($\times 10^3$)
24.2	70
25.1	76
33.5	93
34.5	93
35.0	110
37.2	120
39.1	125
42.0	139
44.8	145
45.0	139
48.0	150
50.0	168

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Source: https://www.industrydocuments.ucsf.edu/docs/xyjh0000

Table IV. Studies of Bacterial Clearance by Animals Housed for Various Periods of Time in The Laboratory Environment.

No. of Days Housed in Laboratory	No. of Bacteria Deposited in Lung	% Bacteria Cleared *
1	107,000	88
7	117,000	86
14	104,000	87
28	86,000	85

*Mean clearance values obtained from single studies.

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Source: https://www.industrydocuments.ucsf.edu/docs/yyk0000

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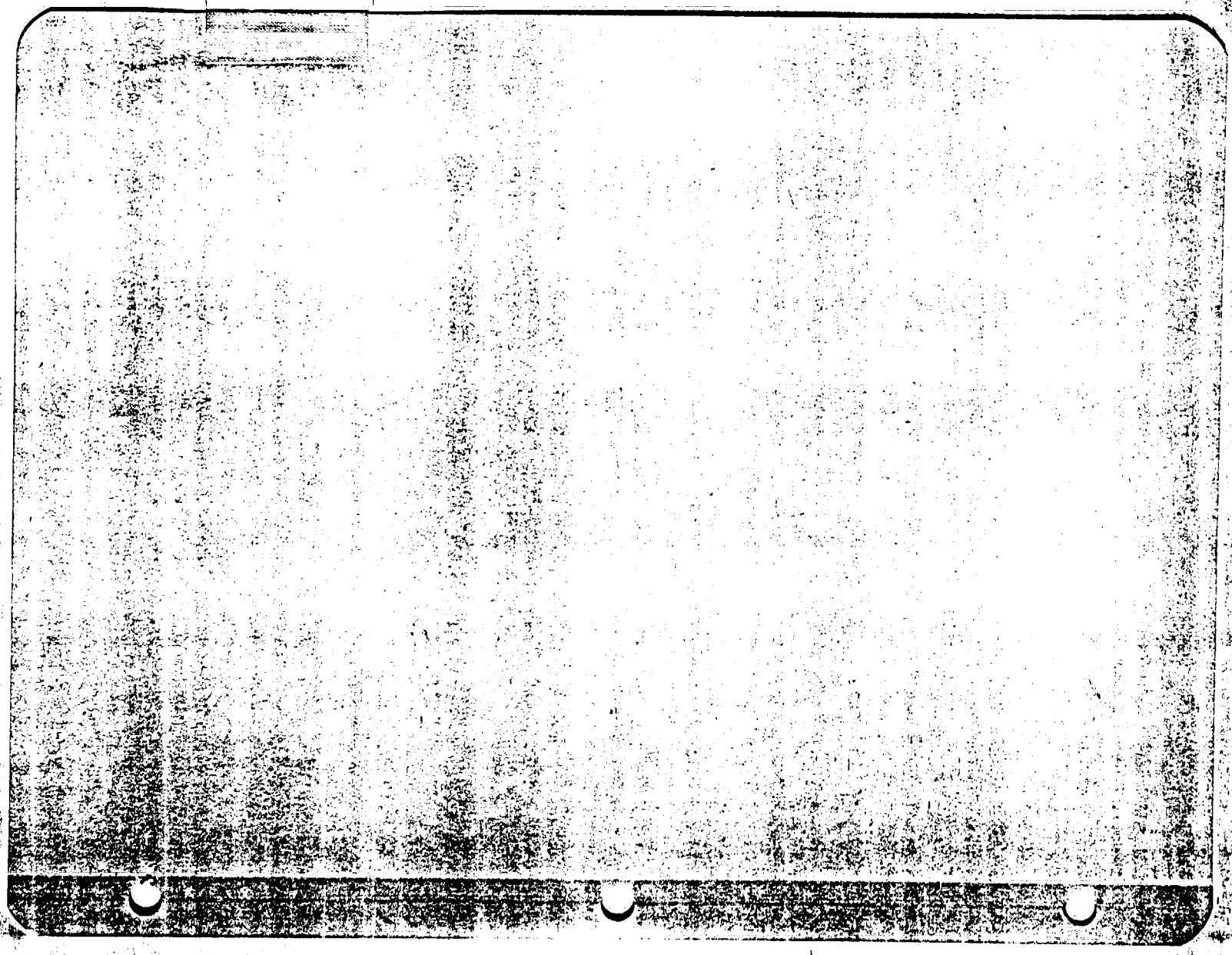

Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

THE COUNCIL FOR TOBACCO RESEARCH – U.S.A., INC.

February 6, 1973

P-1

Grant Application No. 857R1

To: The committee comprising Drs. Gardner, Loosli and Wyatt

Subject: Jerome I. Kleinerman, M.D., St. Luke's Hospital, Cleveland
Renewal application No. 857R1
"Experimental Emphysema: The Effects of Prolonged Dust and Nitrogen Dioxide Exposure on the Physiologic and Morphometric Parameters of the Hamster Lung"

History

This request, for $16,414, is for the second year of an approved two year program (against an initial estimate of $14,835).

The grant now current is for $31,600.

Documents Submitted (attached)

1. Application dated 1/31/73.

2. Progress report, 7-1-72 -- 12-30-72.

F.W.N.

FWN:wg
Encls.

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Source: https://www.industrydocumentslibrary.ucsf.edu/tobacco/xyk0006

Comm.
Dr. Gardner
Dr. Loosli
Dr. Wyatt

CHRONIC PULMONARY DISEASES

#857R1
#857 - 7/1/72 - 6/30/73

The Council For Tobacco Research–U.S.A., Inc.

110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

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FEB 2 1972

Application For Renewal of Research Grant
(Use extra pages as needed)

First Renewal [X] Second Renewal [ ] Date: 1-31-73

1. Principal Investigator (give title and degrees):

Jerome I. Kleinerman, M. D.
Head, Division of Pathology Research & Clinical Pathology, St. Luke's Hospital
Professor of Pathology, Case Western Reserve University

2. Institution & address:

St. Luke's Hospital
11311 Shaker Blvd.
Cleveland, Ohio 44104

3. Department(s) where research will be done or collaboration provided:

Division of Pathology Research
Department of Pathology

4. Short title of study: Experimental Emphysema : The Effects of Prolonged Dust and Nitrogen Dioxide Exposure on the Physiologic and Morphometric Parameters of the Hamster Lung

5. Proposed renewal date: 7-1-73 -- 6-30-74

6. How results to date have changed earlier specific research aims:

No change

7. How results to date have changed earlier working hypothesis:

No change

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Source: https://www.industrydocuments.ucsf.edu/docs/yhy10000

8. Any additional facilities now required? Describe briefly:

None

9. Any changes in personnel? Append biographical sketches of new key professional personnel:

Ronald Reschly, Research Assistant left because of entry into Medical School.

Replacement : Quantimet Technician, Joan Sorensen

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10. Append outline of experimental protocol for ensuing year. (Appended)

11. List publications or papers in press resulting from this or closely related work. (append reprints or manuscripts not previously sent). Niewoehner, D. E. and Kleinerman, J.: Effects of Experimental Emphysema and Bronchiolitis on Lung Mechanics and Morphometry. (Accepted for Publicated) Journal Applied Physiology, 1973

Niewoehner, D. E. and Kleinerman, J.: Pulmonary Mechanics and Morphometry in Postmortem Human Lungs (submitted to American Thoracic Society, Jan. 1973)

Kleinerman, J. and Niewoehner, D. E.: Physiological, Pathological and Morphometric Studies of Long Term Nitrogen Dioxide Exposures and Recovery in Hamsters (submitted to American Thoracic Society, Jan. 1973)

12. Summary progress report (append in standard form as separate document, unless recently submitted).

Source: https://www.industrydocuments.ucsf.edu/docs/xpyd0000

13. Budget for the coming year:

A. Salaries (give names or state "to be recruited")	% time	Amount
Professional (give % time of investigator(s) even if no salary requested)		
Kleinerman, Jerome I. M. D.	20%	none
Technical		
Harold, William, Physiology Technician	25	3,974
Townsend, Betty, Histologist	50	5,842
Sorensen, Joan, Quantimet Tech.	25	3,500
Cannon, Michael, Animal Caretaker	15	1,098
Sub-Total for A		14,414

B. Consumable supplies (by major categories)

Animal Feed
Hostology Supplies
Recording Paper for Physiological Studies

Sub-Total for B 2,000

C. Other expenses (itemize)

Sub-Total for C None

Running Total of A + B + C 16,414

D. Permanent equipment (itemize)

Sub-Total for D None

E. Indirect costs (15% of A+B+C) 2,462

Total request 18,876

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

14. Other sources of financial support:

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Experimental Emphysema : Analysis of Mechanisms	NIH 2R01 ES00264-06A1 National Institutes of Health	40,000/yr	12-1-72 -- 11-30-77
A Correlative Study of Physiologic & Pathologic Findings in Obstructive Pulmonary Disease in Postmortem Lungs	Nat'l Tuberculosis & Respiratory Disease Assoc.	10,587	7-1-72 -- 6-30-73
Evaluation of Heterozygous State for Human Alpha$_1$ - Antitrypsin Deficiency	Ohio Thoracic Society	9,775	7-1-72 -- 6-30-73
Pulmonary Pathology in Cystic Fibrosis	Cystic Fibrosis Foundation	9,359	7-1-72 -- 6-30-73

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Relationship of Emphysema to Pulmonary Surfactant. An In Vitro and In Vivo Study	Ohio Thoracic Society	11,500	7-1-73 -- 6-30-74
A Correlative Study of Physiologic & Pathologic Findings in Obstructive Pulmonary Disease in Postmortem Human Lungs	Nat'l Tuberculosis & Respiratory Disease Assoc.	11,087	7-1-73 -- 6-30-74
Pulmonary Pathology in Cystic Fibrosis	Cystic Fibrosis Foundation	10,309	7-1-73 -- 6-30-74

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name Jerome Kleinerman, M. D.

Signature Jerome Kleinerman Date 1/31/73

Telephone 216 (Area Code) 791-1000 (Number) Ext. 269 (Extension)

Checks payable to

Frank A. Oblak, Controller

Mailing address for check:

St. Luke's Hospital
11311 Shaker Blvd.
Cleveland, Ohio 44104

Responsible officer of institution

Typed Name Charles K. Arter, Jr.

Title President

Signature [signature] Date 1/31/73

Telephone 216 (Area Code) 791-1000 (Number) Ext. 424 (Extension)

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

Procedure For The Ensuing Year

Exposures and and the associated monitoring of NO_2 and dust concentrations will continue for at least 6-8 additional months. An exact decision regarding the termination date will depend upon the health of the exposed animal colony, and local circumstances. In any event exposures will probably not continue past 18 months. At the time of sacrifice aliquots of animals from each of the experimental groups A) Dust only, B) Dust/NO_2 and Controls will be sacrificed at two periods following removal from exposure. One sacrifice period will be immediately following removal; a second will occur from 2-4 weeks after removal. The latter sacrifice is to evaluate the reversibility of any functional or morphologic lesions which may be present. At the time of sacrifice the lungs will be exised and measurement made following the protocol submitted in last years original application. In order not to be repetitious, the detailed protocol for this study will not be repeated. Studies will include static compliance, and dynamic compliance at 18, 45, 72 and 110 cycles per minute. Flow-volume curves during passive and forced deflation (the latter at -50 cm H_2O) will be performed, and expiratory flows, total pulmonary resistance, maximal expiratory flows and upstream resistances will be calculated. The mean values, standard deviations and standard error of the means will be calculated. Analyses of any differences which may be found between means in the various groups will be compared by the student's "t" test and the probability of significance of these data determined.

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After physiologic studies, the lungs will be inflated at a constant pressure of 25 cm. H_2O and prepared for histologic study. This preparation will be carefully performed so that morphometric analyses can be done for calculation of mean linear intercept and internal surface area. In addition histologic study for abnormalities in the lung parenchyma, bronchi and bronchioles will be completed. Additional morphometric analyses will be done utilizing quantimet techniques for evaluaion of goblet cell counts. For these studies tissues must be stained with the Alcian Blue-PAS technique. The extent, location and reaction about the pigment deposits in the lung will be studies to determine if the localization mimics that seen in human emphysema; if the pigment is intra or extracellular and if the cells may which contain pigment have invaded the interstitial tissue or merely exist within the alveolar spaces. The histologic study will be most valuable in determining whether exposure to the low NO_2 concentrations with dust can provoke the broncho-alveolar epithelialization so characteristic of higher levels of NO_2 exposure and if the epithelial papillary proliferation observed with higher doses of NO_2 also is provoked with the lower concentrations used in these studies. Finally the reversibility of the lesions which are seen immediately after sacrifice will be determined by study of hamsters sacrificed at the later period after removal from exposure.

The physiologic data including maximal expiratory flows, static recoil pressures and total pulmonary and upstream resistances will

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be compared between the various exposure groups to determine if exposure to a combination of dust and NO_2 causes greater functional abnormalities than dust alone. The relationship of L_m and ISA to elastic recoil pressure and maximal expiratory flows will be studied in all groups. The objectives of the correlations to be evaluated are to determine the effects of dust alone and in association with a low concentration of NO_2 on the physiologic and pathologic parameters of the lung.

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CTR Grant # 857 Progress Report No. 1

Investigator: Jerome Kleinerman, M. D.

Institution : St. Luke's Hospital

Address : 11311 Shaker Blvd.
Cleveland, Ohio 44104

Inclusive dates covered : 7-1-72 --- 12-30-72

Title

Experimental Emphysema : The Effects of Prolonged Dust and Nitrogen Dioxide Exposure on the Physiologic and Morphometric Parameters of the Hamster Lung

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Progress Report

The project entitled, "Experimental Emphysema : The Effect of Prolonged Dust and Nitrogen Dioxide Exposure on the Physiologic and Morphometric Parameters of the Hamster Lung" has been operational from July 1, 1972. This report covers the period through December 31, 1972. During this period the exposures have been initiated and are in progress. This has entailed development of dust disseminating procedures and methods of measuring dust concentrations simultaneously with the evolution of Nitrogen Dioxide. Three groups of 36 male Syrian hamsters weighing approximately 80 Gm. each and housed separately in gerbil cages have been exposed as follows : Group 1 Exposed to respirable fly ash gathered from the electrostatic precipitators of the Cleveland Electric Illuminating Co. The total dust concentration in chamber A for the six month period is 3.21 mg/cu. m. (S. D. ± 0.96; S. E.±0.39). The respirable dust collected by elutriator during the same period has been 0.84 mg/cu. m. (S. D. ± 0.35; S. E.±0.14). In chamber B the six month mean total dust concentration was 4.35 mg/ cu. m. (S. D. ± 1.24; S. E.± 0.50); the mean respirable dust concentration in this chamber was 0.83 mg/cu. m. (S. D. ± 0.13; S. E.± 0.05). Dust concentrations vary according to the site at which the collection is made. This has been standardized as much as possible by placing the collecting millipore filters and elutriators at sites of comparable dust concentration and maintaining these collection sites continuously. Samples are collected five days per week for periods of 4-6 hours; exposures are performed seven days per week.

The mean Nitrogen Dioxide concentrations during the first six months is 2.07 ppm (S. D. ± 0.38; S. E. ± 0.15). Nitrogen Dioxide concentrations are recorded continuously by mast meter on a Honeywell-Brown recorder. Nitrogen Dioxide exposures are performed seven days weekly for 20-22 hours daily. The mast meter readings are calibrated twice daily Monday through Friday and once only on Saturday and Sunday. Calibration is by the Saltzmann method of Nitrogen Dioxide analysis.

The mean weight of the animals exposed to dust and Nitrogen Dioxide, Group 2 has increased from 74 ± 7 Gm. to 124.5 ± 14 Gm. in a six month period. In the same period hamsters exposed to dust only have gone from their original mean weight of 77.1 ± 6.83 Gm. to 124.76 ± 12.55 Gm. The values for the control (unexposed) group, Group 3, are as follows : Mean initial weight 80 ± .9 Gm; at the end of six months 124.2 ± 12.54. Thus the exposures do not seem to have interfered with the usual growth and weight increase as compared with the controls.

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A number of animals have died of a variety of causes. Several have developed what appears to be abscesses in sinuses, orbit and maxilla which progress to intracranial pyogenic infections either meningitis or subdural abscesses. On several occasions cultures taken at autopsy have demonstrated a variety of organisms including E. Coli. Pseudomonas, anerobic streptococci and monilia. Other causes of death include nephrosis and anasarca, or trauma. In other cases cannabalization prevented autopsy study. The mortality during the six month period of the experiment has been none in the dust group, seven in the dust Nitrogen Dioxide exposures and six in the control.

Preliminary experiments have been performed in order to develop and perfect the methods of physiologic study. In a series of normal control animals the following physiologic data has been collected.

Ambient Air : n = 9, Static compliance = .526 ml/cm H_2O

Dynamic compliance at 20 cpm .295 cc/cm H_2O
50 cpm .266 cc/cm H_2O
80 cpm .250 cc/cm H_2O

Studies during natural deflation :

Expiratory flow rate at 2 cc lung volume (cc) 14.1 ± 2.52, S. E. 0.89

1 cc lung volume (cc) 7.89 + 1.59, S. E. 0.56

Total pulmonary resistance at 2 cc 1.88 cm H_2O/ml/sec ± .62 S. E..23

at 1 cc 1.90 cm H_2O/ml/sec ± .48 S. E. .18

Studies during forced deflation at -50 cm. water:

Max. Expiratory flow rate at 2 cc lung volume 34.78 ± 6.16 SE (2.05)

at 1 cc lung volume 15.32 ± 5.34 SE (1.78)

Upstream resistance at 2 cc lung volume 1.24 ± .40 SE (0.13)

at 1 cc lung volume 1.52 ± 0.72 SE (.24)

These studies indicate that the methods for measurement of postmortem pulmonary function are reliable and reproducible for our studies.

Measurement of mean linear intercept, and internal surface area of the lung has been performed in a series of control animals. These measurements performed by human eye have the following values : Mean Linear Intercept (L_m) .0718 ± .0030 mm and internal surface area (ISA) 2,330 ± 390 cm^2. In addition efforts have been initiated to develop automated techniques for measurement of L_m and ISA. The quantimet image analyzing computer has been utilized for these measurements. In a series of comparisons the L_m and ISA as measured by quantimet at a magnification of 80 times were L_m .0685/mm and ISA 2760 cm^2 while at a magnification of 200 times measurement values were L_m .0601/mm^2 and ISA 3150 cm^2, as compared to values measured by human eye in the same animal lung of L_m .0678/mm and ISA 2,650 cm^2.

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

These data suggest that the morphometric evaluation of the hamster lungs may be adaptable to semi-automated techniques using the Quantimet. This could be a considerable boon in terms of time saving and avoidance of terribly tedious and exacting measurements.

Preliminary measurements of ISA have been performed in this laboratory by the manual method described by Thurlbeck and compared to values obtained by an automated method using the Quantimet 720. The procedure used for comparing these two methods is as follows : A histologic slide of lung tissue (6 micron) stained with hematoxylin-eosin was overlayed with a grid of 2 mm squares. The grid allows for systematic scanning of the section without inadvertently duplicating fields for measurement. By using machine modules which house a shading corrector and a module capable of projecting a precalibrated variable measuring frame, alveolar wall intercepts were machine counted.Manual intercept counts were performed on the projected image on the television monitor from two of the five projected precalibrated lines. Fifty random fields were counted avoidingareas which con-contained large vessels and bronchi. An analysis of variance was applied to test for significant differences between machine and manual counts on the television screen. When 50 automated counts were compared to the manual counts the p value is <.60, no significant difference. When 50 frame manual counts on the TV monitor are compared to 125 machine counts which included the same field, the p value was <.90 again indicating no significant difference but a closer association of values. Since the time required to increase the number of quantimet measurements is insignificant, the protocol will stipulate 125 variable frame counts measured.

Staining procedures and Quantimet 720 measurement methodologies have also been devised for the counting of goblet cells and for determining the ratio of total (area) gland mass to total area of the tracheal or bronchial section. Histologic sections (6 micron) stained with Alcian blue and Schiff reagent (PAS) will be used to count goblet cells, accumulate the area positively stained by the Alcian blue - PAS. These data will be related to the total area measurement of the section. Tracheal and bronchial sections stained with Alcian blue and with PAS will be used to measure the ratio of total area of gland mass to total area of the section. This will be accomplished utilizing the light pen module of the Quantimet 720, which can measure and accumulate data on isolated features in the optical field.

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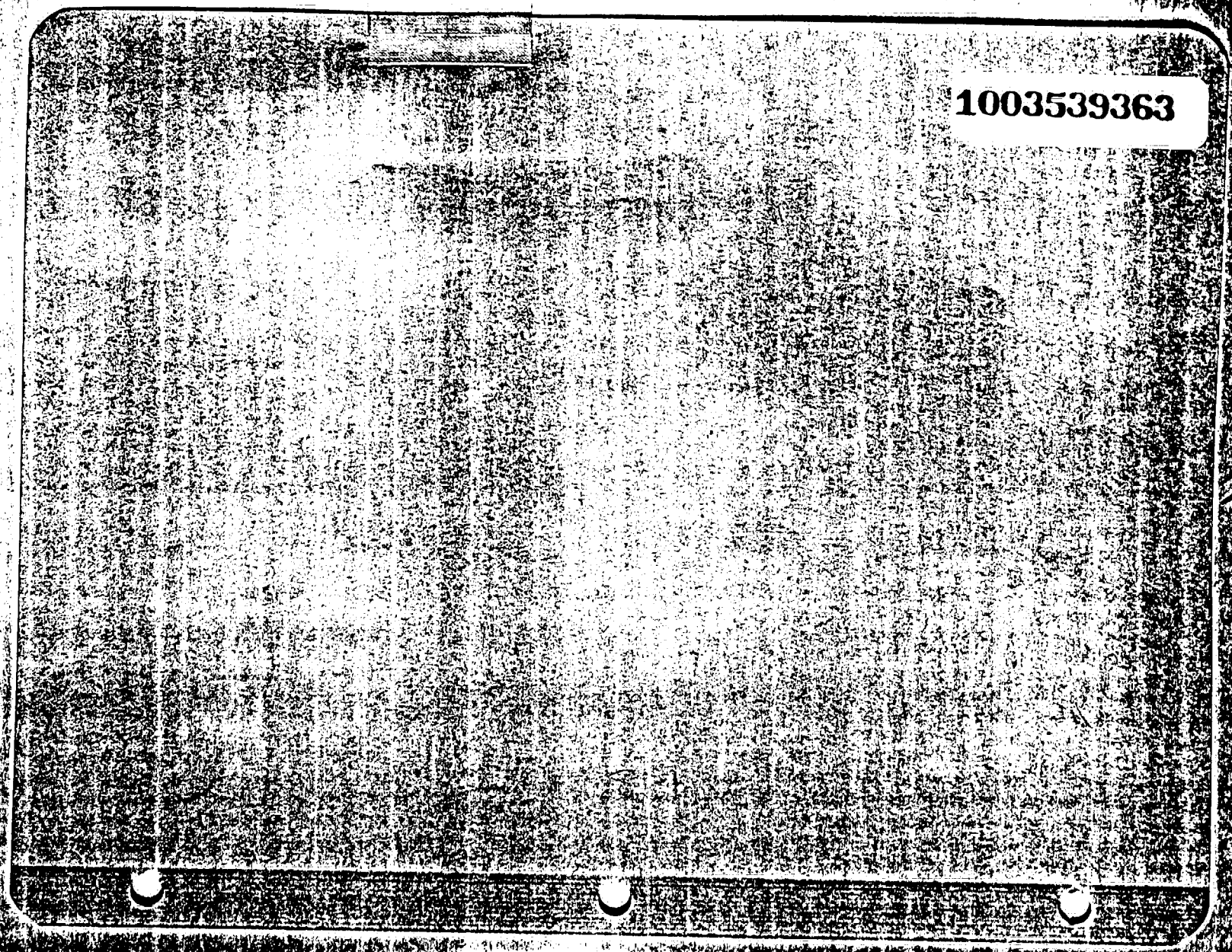
#573BR2 LOOSLI



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THE COUNCIL FOR TOBACCO RESEARCH–U.S.A., INC.

January 30, 1973 P-4

Renew for 9 mo effective Oct 1, 1973 at the same rate. Terminate June 30, 1974

Grant Application No. 573BR2

To: The committee comprising Dr. Gardner, Jacobson, Meier, and Sommers

Subject: Clayton G. Loosli, M.D., Ph.D., University of Southern California, Los Angeles
Renewal application No. 573BR2
"The effects of Fresh Cigarette Smoke Inhalation on the Respiratory Tract of Mice"

History

As you know, this line of investigation has been supported by CTR since 1966.

The request is for approximately $182,000. Dr. Loosli earlier estimated for this year $112,000. He states his reasons for suggesting expansion of the program at this time.

Document Submitted

Attached is a copy of the application dated January 24, 1973.

Dr. Loosli says a progress report will be provided early in February. At that time a copy will be forwarded to you.

FWN

F.W.N.

FWN:wg
Encl.

Vinc Lisanti says they are far behind in examining histological specimens. Should finish this instead of playing around with virus identification

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

Comm.
Dr. Gardner
Dr. Jacobson
Dr. Meier
Dr. Sommers

CHRONIC PULMONARY DISEASES
RENEWAL PROPOSAL

#573BR2
#573BR1-10/1/72 - 9/31/73
#573B -10/1/71 - 9/31/72
Contract # 7 - 10/1/70 - 9/31/71
573A-10/1/69 - 9/31/70
573-9/1/66 - 9/31/69

The Effects of Fresh Cigarette Smoke Inhalation on the Respiratory Tract of [illegible]

RECEIVED
JAN 29 1973
SUBMITTED TO

COUNCIL FOR TOBACCO RESEARCH - U.S.A.
110 East 59th Street, New York, N.Y.
10022

BY

UNIVERSITY OF SOUTHERN CALIFORNIA
University Park
Los Angeles, California 90007

Principal Investigator:

Clayton G. Loosli, M.D., Ph.D.
Hastings Professor of
Pathology

Approved for the
Department of Pathology:

Nancy E. Warner, M.D.
Chairman, Department of
Pathology

Approved for the University by:

Zohrab A. Kaprielian,
Vice President, Academic
Administration and Research

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Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A., INC.

110 East 59th Street
New York, N.Y. 10022
(212) 421-6534

Application for Renewal of Research Grant
(Use extra pages as needed)

First Renewal Second Renewal Date: Jan. 24, 1973

1. Principal Investigator (give title and degrees):
   Clayton G. Loosli, M.D., Ph.D. Hastings Professor of Medicine and Pathology
   Helgard Niewisch, D.V.M., Research Associate in Pathology
   Kuen-Shan Hung, Ph.D., Assistant Professor of Anatomy and Pathology.

2. Institution and address:
   The University of Southern California
   University Park
   Los Angeles, California 90007

3. Department(s) where research will be done or collaboration provided:
   USC School of Medicine
   Department of Pathology
   2025 Zonal Avenue
   Los Angeles, California 90033

4. Short title of study:
   Effects of Fresh Cigarette Smoke Inhalation on the Respiratory Tract of Mice

5. Proposed renewal date:
   July 1, 1973

6. How results to date have changed earlier specific research aims:
   Basically, there are no changes in the objectives set forth in the application two years ago. However, in order to concentrate solely on the effects of inhalation of fresh tobacco smoke alone, the "synthetic smog" exposure studies will be discontinued as of June 30th. The SS exposure room will be used as an additional filtered air room where the additional smoke exposed and control mice will be housed.

7. How results to date have changed earlier working hypothesis:
   There is no basic change in working hypothesis. Four strains of mice are being exposed and sacrificed periodically and the lungs examined histologically, histochemically, and by E.M. procedures. Current findings strongly suggest that a C type virus particle is responsible for bronchial epithelial metaplastic growth. What activated the virus is not known. Studies will be directed toward identification of the viral agent or

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Source: https://www.industrydocumentslibrary.ucsf.edu/tobacco/ytyf0000

or agents, and transmission of virus in SPF mice under strict conditions of isolation. Also macrophage accumulation and retention in the lungs of smoke exposed animals suggests that they may contribute to lung tissue breakdown. Studies will be directed toward ellucidating the role of pulmonary macrophages in the development of emphysematous-like lung lesions.

8. Any additional facilities now required? Describe briefly:
No additional facilities or equipment except a CO_2 incubator is required. As is the case now, the Air Pollution Building will continue to be used for exposing and housing both smoke exposed and control mice. Synthetic smog (SS) studies will be discontinued. The SS room supplied with filtered air will be used to house the sham and non-smoking control animals. For viral transmission studies, the germfree isolator units in the germfree facility will be employed to house the mice inoculated with suspected infectious material. Tissue culture facilities are available for culture of lungs and other organs of mice suspected of harboring virus. Serological procedures for identifying antibodies to suspected viruses will be employed in identifying the virus.

9. Any changes in personnel? Append biographical sketches of new key professional personnel:
Dr. Bernard Hanes, not listed on the grant, will continue to serve as a consultant. Dr. Hung continues to spend essentially all his time on electron microscopic examination of lung tissue. Dr. Helgard Niewisch, D.V.M., who joined our group last September, will continue to supervise the smoke exposure program and the smoking machine operators - Paul LeVangie, Peter Bondante, and Mrs. Alicia Navarro. The smoking machine operators are also being trained in various laboratory procedures, to clean, wrap and sterilize bacteriological and virological equipment and to prepare the food for the germfree mice. They will make valuable assistants when the studies on the nature and identification of the virus begin.

Dr. Niewisch, having been in the Department of Microbiology for 6 years, has competence in bacteriological and virological procedures. She will participate in the studies associated with isolation and identification of the virus seen in the E.M. photographs, in association with the epithelial metaplastic growth. Drs. Richard O'Brien and John Parker have an active tissue culture laboratory and have offered to assist us and train, if necessary, the virologist, which at this point in time, has to be recruited.

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Source: http://www.industrydocuments.ucsf.edu/docs/ytyf0000

John Hardy, M.S. (Exp. Path.), Sherman Stinson, M.S. (Exp. Path.), and Jeanne Joyce, B.S., Germfree laboratory supervisor (not listed on grant), will have responsibility of the animal transmission studies and autopsies of all animals. Mr. Stinson, a trained biochemist, will carry out the phospholipid and surfactant analysis on the lungs.

While the salaries of the technical staff may seem somewhat high, Misses Hertweck, Stone and Serebrin, and the animal caretakers have been with our group for several years. Their salaries are comparative with others in the University with similar skills and experience.

9A. Justification of budget:

For the past 5 years we have had partial support from the E.P.A. for study the effects of "synthetic smog" on the respiratory tract of mice. This grant will terminate March 31, 1973. Also for the past 5 years, the N.I.H. (Laboratory Facilities Division) has underwritten the Germfree Facility. Support for the Germfree laboratory will terminate May 31st. Continued support for our experienced personnel, who have been partially supported by the above government organizations in the past, is absolutely essential for the performance of the studies of the effect of smoke inhalation on the respiratory tract of mice.

No other grant support or applications are pending. Through the Hastings Foundation and the Hughes Employees Give Once Club, the University is contributing significantly in the support of these studies. It will be a great advantage to this study not to have it superimposed on the synthetic smog study. It will aid significantly in planning to have a commitment for an additional two years at the level requested plus 5 percent for living cost increases.

10. Append outline of experimental protocol for the ensuing year:

Since June, 1972, the CTR-Walton Horizontal Smoke Exposure Machines have been in use. At the present time, four are in operation. Four different strains of mice have been employed: CD-1 white, pathogen free (SPF), Ajax white non-pathgen free, C-57 Black non-pathogen free, and Snell non-pathogen free mice. A total of 500 mice are being exposed to fresh cigarette smoke daily. Half will be sacrificed in February and the other half in May, 1973. Synthetic smog studies will be discontinued as of May 31st, 1973.

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OUTLINE OF STUDIES FOR 1973-74

A. Continuation of exposure of mice to fresh cigarette smoke from 1A1 low nicotine reference cigarettes. We hope to continue to use the above strains of SPF mice which have been

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caesarian derived and barrier maintained to avoid the problem of respiratory infections among the mice. Based on our experience the past year, the maximum daily exposure, which will allow long term survival, will be exposure to fresh smoke from three simultaneously "fired" 1A1 cigarettes once a day. The exposure time after a two-second puff will continue to be 21 seconds, with a purge time of 39 seconds. The Ajax and the CD-1 strains appeared to be somewhat less tolerant and are receiving smoke from two cigarettes a day for 5 days a week. The smoke exposed animals on a once a day regimen maintain their weight. As our interest is primarily in lung changes due to smoke inhalation, we hope to continue to use the low nicotine cigarettes to maintain the maximum exposure up to the limit of tolerance.

The two large filtered air rooms in the air pollution building will be employed to house the mice. One will house the animals subjected to smoke and the other will house the sham and non-sham controls. Sufficient animals from each mouse group will be used so that 18 from each group will be sacrificed at intervals of 3 months. As mice are sacrificed, additional mice of the same age will be added to the smoking regimen. It is our impression so far, that increasingly older mice become less tolerant of fresh cigarette smoke inhalation.

Eighteen animals from the smoke exposed and control groups are chosen to be sacrificed, for it represents the maximum number which can be subjected to smoke inhalation at one time. Several procedures for examining the lungs and other organs will be carried out, as are now being done. Eight mice will be sacrificed for light and electron microscopic study, three for histochemistry, and seven for phospholipid and surfactant analysis. Before sacrifice, all mice will be bled out and the sera stored for immunological studies. Portions of the lung for phospholipid and histochemical analysis will be quickly frozen for subsequent virological analysis. Animals becoming ill during the course of the study will be bled and sacrificed in order to obtain fresh tissue for virological studies.

B. Study of the pulmonary macrophage reaction in Snell mice. Studies thus far indicate that mice inhaling whole fresh smoke from 1A1 reference cigarettes accumulate large foamy macrophages in the pulmonary alveoli in the region of the terminal bronchioles and that these macrophage collections are still present at least one month after smoke exposure is discontinued. It is thought by some that pulmonary macrophages may secrete enzymes which cause lung tissue breakdown, leading to emphysema.

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Source: https://www.industrydocuments.ucsf.edu/docs/qyfb0000

It is assumed that the macrophage reaction in the whole smoke exposed mice is due to the particulate component of smoke. To test this, the following experiment will be carried out: Three groups of Snell mice will be employed. One group will be exposed to fresh whole smoke; one to the gas phase of smoke only, after passing through a Cambridge filter; the third group will serve as controls.

The smoke exposed group will be subjected to a heavy smoking regimen, so that the macrophage reaction can be expected to be present after three months of smoking. Smaller numbers from the three groups will be sacrificed and their lungs examined for the presence of macrophage collections. Others will continue to be exposed to whole and gas phase smoke for another 3 months. At the same time, some animals exposed to whole smoke will be set aside and sacrificed at later periods to note the continued presence of the macrophages in the lungs.

C. Identify the role of viral agent or agents associated with bronchial epithelial metaplasia: During the course of regular smoking schedule of the four strains of mice the latter part of October, 1972, animals from all groups became ill and some died. A sacrifice after 5 and 5 1/2 months revealed widespread pulmonary lesions in all groups - smokers and non-smokers living in the SS and filtered air rooms. The reconstruction of the pathogenesis of the lesions suggests a virus infection of some kind, although no known infected animals or viral agents had ever been introduced into the chambers. The microscopic appearances of the lung lesions were similar in the four strains of mice whether they had been exposed to cigarette smoke or not.

A detailed study of the lung lesions in the Snell mice has been made.

The early lesions show marked destruction of the bronchial epithelium, quite similar to that seen in influenza virus infections. On recovery, the regenerating epithelial membranes become markedly thickened and metaplastic and grow peripherally into the alveolar ducts and associated alveoli as squamous cell masses. The inflammatory cells in the early lesions are polymorphonuclear leukocytes. These become replaced with mononuclear cells in the older lesions. The epithelial lesions are focal in character, involving only a few bronchi with the remaining lung having a normal appearance. Older lesions show degeneration of the squamous cell masses, leaving the alveoli lined with low cuboidal epithelial cells, which stain specifically with G1-6-PDH.

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Examination of the lung lesions from one smoke exposed mouse and one unexposed mouse from the Snell group revealed C type viral particles budding from the membranous surfaces of the cells.

It seems certain to us that this C type virus particle is basically involved as the inciting agent in the production of the epithelial growths in the lungs. As all the different groups of animals became ill at the same time whether they were smoke exposed or not, suggests that the virus was disseminated from one group to another as an airborne infection. Undoubtedly the virus is probably still active in the mouse colonies.

The following studies will be attempted:

a.) Rescue of virus through animal to animal transmission. Quickly frozen lung tissue from animals ill with the infection will be ground up and the supernate (hopefully) containing the active virus will be given intranasally to SPF Snell and CD-1 SPF mice which will be kept in Germfree isolators to prevent cross infection.

b.) There will be an attempt to grow the virus in cultures of mouse lung tissue from young SPF Snell and CD-1 mice. Cultures will be examined with the aid of the electron microscope. Drs. O'Brien and Parker have offered to assist in the cell culture preparation of virus activity and serological examinations.

c.) If the virus can be transmitted horizontally in SPF mice and lesions described above reproduced, the agents will then be given to Vitamin A deficient mice and mice receiving carcinogenic compounds.

d.) If the virus can be grown, permission will be requested to send it to Microbiological Associates for identification.

INTERVAL STUDIES

a.) To continue smoke inhalation studies in the current non-pathogen free Ajax, C-57Bl, Snell, and CD-1 mice.

b.) Continue the examination of infected lung tissues from the Ajax, C-57Bl, Snell, and CD-1 smoke exposed and non-exposed groups.

c.) Look for and attempt to isolate the virus in animals to be sacrificed in February and May, 1973.

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Source: https://www.industrydocuments.ucsf.edu/tobacco/

11. List publications or papers in press resulting from this or closely related work. (append reprints or manuscripts not previously sent)..

Loosli, C.G., Buckley, R.D., Hertweck, M.S., et al. Pulmonary Response of Mice Exposed to Synthetic Smog. Annals of Occupational Hygiene; In Press.

Loosli, C.G., Buckley, R.D., Hwang-Kow, S.-Y., et al. Experimental Airborne Influenza PR8-A Infections in Germfree Mice. IV International Symposium on Germfree Research. Academic Press, New York; In Press.

Loosli, C.G., Buckley, R.D., Hwang-Kow, S.-Y., et al. Effect of Air Pollutants on Resistance of Mice to Airborne Influenza A Virus Infection. IV International Symposium on Aerobiology, Utrecht, the Netherlands, Sept. 6, 1972; In Press.

Loosli, C.G., Stinson, S.F., Hwang-Kow, S.-Y., et al. Effect of Vitamin A Intake on the Pathology of Airborne Influenza A Virus Infection. IV International Symposium on Aerobiology, Utrecht, the Netherlands, Sept. 6, 1972; In Press.

Hung, Kuen-Shan, Hertweck, M.S., Hardy, J.D., and Loosli, C.G. Innervation of Pulmonary Alveoli of the Mouse Lung: An Electron Microscopic Study. American Journal of Anatomy 135: 447-495, 1972. Reprint sent to C.T.R.

Hung, K.-S., Hertweck, M.S., Hardy, J.D., and Loosli, C.G. Filaments in Fibroblast in Pulmonary Alveolar Wall. Proceedings, 30th Annual Meeting of the Electron Microscopy Society of America, pp. 248-249. C.J. Arceneaux, editor. Claitor's Publishing Division, Baton Rouge, 1972. Reprint sent to C.T.R.

Hung, K.-S., Hertweck, M.S., Hardy, J.D., and Loosli, C.G. Electron Microscopic Observations of Nerve Endings in the Alveolar Walls of Mouse Lungs. Abstract appeared in Amer. Rev. Resp. Dis., 1972 105, 1008. Accepted for publication by the Amer. Rev. Resp. Dis. January, 1973. Reprint sent to C.T.R.

Hung, K.-S., Hertweck, M.S., Hardy, J.D. and Loosli, C.G. Ultrastructure of Nerves and Associated Cells in Bronchiolar Epithelium of the Mouse Lung. Submitted for publication to the Journal of Ultrastructure Research. Reprint sent to C.T.R.

12. Progress report submitted separately.

1003539372

Source: https://www.industrydocuments.ucsf.edu/docs/yyjb0000

13. Budget for the coming year:

A. Salaries (give names or state "to be recruited")
Professional (give % time of investigator(s) even if no salary requested)

	% Time	Amount
Professional:		
Clayton G. Loosli, M.D., Ph.D. Principal Investigator	80*	$16,350
Helgard Niewisch, D.V.M., Res. Assoc. Path.	100	14,770
Kuen-Shan Hung, Ph.D., Asst. Prof. Anat&Path.	30**	1,470
Virologist - to be recruited	100	11,000
Technical:		
M. Sue Hertweck, M.S., Electron Microscp.	100	11,330
Edna Stone, M.S., Histology Tech.	100	11,520
Rhoda Serebrin, B.S., Histochemist	66	6,750
Lee Strom, B.S., E.M. Tech	100	6,960
Pete Radanovich, Animal Caretaker	100	5,530
Carlos Garcia, Animal Caretaker	100	8,860
Smoking Machine Operators (3)	100	20,680
		115,220
Fringe Benefits (10%)		11,522
Sub Total for A		$126,742

B. Consumable supplies (by major categories)

Animals: 2,400 mice at $1.00	2,400
20 rabbits at $4.50	90
Food and Vivaria costs:	6,000
Electron microscopic supplies	2,500
Histopathological, Histochemical supplies	1,500
Microbiological, Virological, Immunological supplies	3,000
Reference cigarettes	650
Sub Total for B	13,240

C. Other expenses (itemize)

Maintenance of electron microscope	2,400
Service and Repairs of Equipment	2,000
Maintenance of Air Pollution Bldg. & Germfree Facility	4,000
Office and Communication costs	2,000
Publication costs	1,500
Travel, two meetings East Coast, two local meetings	1,200
Sub Total for C	$13,100
RUNNING TOTAL OF A+B+C	$156,082

D. Permanent Equipment (itemize)

CO_2 Incubator National	2,500
Sub Total for D	$2,500

E. Indirect costs (15% of A+B+C) E $23,412

TOTAL REQUEST: $181,994

* 80% effort, but only 28% salary support
** 30% effort, but only 8% salary support

1003539373

Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

14. Other sources of financial support:
List financial support from all sources, including own institution, for this and related research projects:

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
USC School of Medicine Contribution	Hastings Foundation 23-5120-4092	$43,000	7-1-73 to 6-30-74
	Hughes Employees Give Once Club 23-5120-4234	$26,500	7-1-73 to 6-30-74

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
NONE	NONE		

10035393?4

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal Investigator

Name Clayton G. Loosli, M.D

Signature [signature]

Telephone (213)225-1511 x23[illegible]

Checks payable to:
University of Southern California
ATTN: Clark A. McCartney

Mailing address for check:

University of Southern California
University Park
Los Angeles, California 90007

Responsible officer of institution:

Name Dr. Z.A. Kaprielian

Title V.P. Academic Administration and Research

Signature

Telephone (213) 225-1511, x432

Source: https://www.industrydocuments.ucsf.edu/docs/yyyy0000

1003539375

#862R1 TRAVIS

Source: https://www.industrydocuments.ucsf.edu/docs/sgtl0000

THE COUNCIL FOR TOBACCO RESEARCH – U.S.A., INC.

January 31, 1973

P-1

Grant Application No. 862R1

To: The committee comprising Drs. Bing, Loosli and Sommers

Subject: James Travis, Ph.D., University of Georgia, Athens
First renewal No. 862R1
"Biochemistry of Chronic Obstructive Lung Disease"

History

Support is requested for year II of a three year project approved by SAB.

The request is for $22,237 against an earlier estimate for this year of $21,165.

Document Submitted (attached)

Application dated January 24, 1973 including summary progress report.

Comment

Dr. Travis has been asked for copies of the manuscripts identified under Item 11, page 2 of the application. He offered some resistance on the basis of confidentiality, but upon being reassured, undertook to provide one or both of these. They will be forwarded to you when received.

FWN

F.W.N.

FWN:wg
Encl.

1003539376

Source: https://www.industrydocuments.ucsf.edu/docs/wyjd0000

Comm.
Dr. Bing
Dr. Loosli
Dr. Sommers

CHRONIC PULMONARY DISEASES

#862R1
862 - 7/1/72 - 6/30/73

THE COUNCIL FOR TOBACCO RESEARCH—U.S.A., INC.

110 EAST 59TH STREET
NEW YORK, N.Y. 10022
(212) 421-8885

Application For Renewal of Research Grant
(Use extra pages as needed)

First Renewal ☒ Second Renewal ☐

Date: Jan. 24, 1973

RECEIVED
JAN 29 1973

1. Principal Investigator (give title and degrees):

James Travis, Ph.D. Associate Professor

2. Institution & address:

Department of Biochemistry
University of Georgia
Athens, Georgia 30601

3. Department(s) where research will be done or collaboration provided:

Department of Biochemistry, College of Arts and Sciences

4. Short title of study:

Biochemistry of Chronic Obstructive Lung Disease

5. Proposed renewal date: July 1, 1973

6. How results to date have changed earlier specific research aims:

The preliminary results obtained indicate that more than one hydrolytic enzyme is probably responsible for the onset of this type of emphysema. Thus, we are now studying more than one enzyme in order to determine the means by which lung degradation occurs.

7. How results to date have changed earlier working hypothesis:

The hypothesis remains the same; that is, degradation of lung tissue by hydrolytic enzymes occurs because of the lack of alpha-1-antitrypsin in certain individuals.

1003539377

Source: https://www.industrydocuments.ucsf.edu/docs/yrgm0000

8. Any additional facilities now required? Describe briefly:

The very heavy use of chromatographic equipment for the purification of both protease inhibitors from plasma as well as hydrolytic enzymes from leukocytic granules makes the purchase of another fraction collector and a UV monitor of the utmost necessity.

9. Any changes in personnel? Append biographical sketches of new key professional personnel:

Not Applicable

10. Append outline of experimental protocol for ensuing year.

11. List publications or papers in press resulting from this or closely related work. (append reprints or manuscripts not previously sent).

1. Properties of an Inactive Form of Human alpha-1-antitrypsin Purified by Affinity Chromatography. R. Pannell and J. Travis
Federation Proceedings, 1973. (Abstract)

2. Human Pancreatic Enzymes: Isolation and Properties of a Second Form of Human Trypsin. P. Mallory and J. Travis
Biochemistry (submitted for publication)

3. Human Pancreatic Enzymes: Purification and Properties of an Elastase-like Enzyme (Pseudoelastase). P. Mallory and J. Travis
Biochemistry (submitted for publication)

12. Summary progress report (append in standard form as separate document, unless recently submitted).

10035393378

Source: https://www.industrydocuments.ucsf.edu/docs/tyhd0000

13. Budget for the coming year:

A. Salaries (give names or state "to be recruited")	% time	Amount
Professional (give % time of investigator(s) even if no salary requested)		
James Travis, Associate Professor	40	-0-
Research Associate (to be named)	100	8000
Fringe Benefits (14%)		1120
Technical		
Joanne Betso	50	3500
Fringe Benefits (14%)		490
Sub-Total for A		13110
B. Consumable supplies (by major categories)		
Glassware		1500
Chemicals		1000
Biological Materials		500
Sub-Total for B		3000
C. Other expenses (itemize)		
Travel (Foreign-700; Domestic-200)		900
Publication Costs		500
Sub-Total for C		1400
Running Total of A + B + C		17510
D. Permanent equipment (itemize)		
Isco UV Monitor and Accessories		1500
Isco Fraction Collector		600
Sub-Total for D		2100
E. Indirect costs (15% of A+B+C)		2627
Total request		22237

10035393826

Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

14. Other sources of financial support:

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Proteolytic Enzymes of the Blood and Pancreas	NIH HL14778	22957	Jan 1/73 - Dec 31/73
Proteolytic Enzymes of the Blood and Pancreas	NIH Career Development Award 1K04 HL70264	20410	July 1/72 - June 30/73

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name James Travis

Signature [signature] Date 1/23/73

Telephone 404 (Area Code) 542-1334 (Number) 25 (Extension)

Checks payable to

The University of Georgia

Mailing address for check:

Acting Vice President for Business and Finance

The University of Georgia

Athens, Georgia 30602

Responsible officer of institution

Typed Name Robert C. Anderson

Title Vice President for Research

Signature [signature] Date 1/24/73

Telephone 404 (Area Code) 542-3360 (Number) (Extension)

10035393B0

Source: https://www.industrydocuments.ucsf.edu/docs/yvdb0000

10. Experimental Protecol for Coming Year

During the coming year a detailed survey of all of the proteolytic enzymes present in granules will be made as well as the effects of both alpha-1-antitrypsin and alpha-2-macroglobulin on their activities. The continued isolation of the elastaese component(s) will be made and the properties of the enzyme(s) studied. It is difficult at this stage to attribute a great significance to leukocytic elastase(s) as the primary enzyme(s) responsible for lesions in the basement membrane of the alveolar wall. The amount of activity seems too low to account for this digestion and the fact that alpha-2-macroglobulin is still present in alpha-1-antitrypsin deficient patients would suggest that at least some of the elastase activity would be removed by this protein. It may well be that several enzymes are acting synergistically to cause the digestion of the alveolar membrane or that a proteolytic enzyme, not inhibitable by alpha-2-macroglobulin but inhibitable by alpha-1-antitrypsin, is responsible.

A shift to the isolation of alpha-1-antitrypsin by the techniques described in section 12 will be made, which should result in the isolation of at least the two most important forms of this protein, type MM and type ZZ. The comparative properties of each enzyme inhibitor will be studied to determine what differences might account for the low concentration of type ZZ alpha-1-antitrypsin in plasma.

1003539381

Source: https://www.industrydocuments.ucsf.edu/docs/yfyj0000

12. Summary Progress Report

The objectives of this project were to isolate human leukocytic elastase as well as human alpha-1-antitrypsin and determine the relationship of these proteins to each other as well as their role in the onset of chronic obstructive lung disease. In the past six months since this project was initiated we have succeeded in purifying to homogeneity an inactive form of human alpha-1-antitrypsin which we intend to use to study some of the physical and chemical properties of this protein. Furthermore, this protein is also being used to produce antibodies which will be used for the preparation of the active form of the inhibitor by affinity chromatography More recently we have found that by using a sepharose-blue dextran derivative we can bing virtually all of the albumin in plasma to this material. Other plasma proteins bind as well but alpha-1-antitrypsin and alpha-2-macroglobulin pass directly through a column of this material without loss of activity. In fact, using plasma which has been passed through a column of Sephadex G-75 prior to passage through the sepharose-blue dextran column, we have been able to isolate fractions containg only two components, one of which is alpha-1-antitrypsin, and the other probably orosomucoid. we feel it should be a simple matter of using ion-exchange chromatography on DEAE-cellulose to isolate the active alpha-1-antitrypsin component in pure form. This technique should also be useful for the isolation of alpha-1-antitrypsin from the plasma of type ZZ individuals who have presented the symptoms of chronic obstructive lung disease. The removal of the albumin fraction will allow the concentration of large volumes of plasma fractions so that the alpha-1-antitrypsin (type ZZ) may be isolated. The technique should have even more far reaching implications for the isolation of trace proteins in plasma heretofore contaminated by the large quantities of albumin normally found in plasma.

The purification of leukocytic elastase to homogeneity on a large scale has been hampered by the strong binding of nucleic acids and hemoglobin to the enzyme, resulting in its inactivation. we have overcome these problems by a) working exclusively with leukocytic granules, b) fractionating the granule extract between 0-60% with $(NH_4)_2SO_4$, c) chromatographing on DEAE-cellulose, d) chromatographing on Sephadex G-75. This last step results in the separation of two proteolytic components both of which appear to be elastolytic in nature. However, neither component is homogenous at this stage.

1003539382

Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

1003539383

MISCELLANEOUS

Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

#826R2 DAWBER

10035393B4

Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

THE COUNCIL FOR TOBACCO RESEARCH – U.S.A., INC.

January 31, 1973

P-1

Grant Application No. 826R2

To: The committee comprising Drs. Jacobson, Loosli and Sommers

Subject: Thomas R. Dawber, M.D., Boston University Medical Center, Boston, Massachusetts
Second renewal No. 826R2
"Smoking Classes, Risk Factors and Cardiovascular Disease"

History

Support is requested for the third and final year of a three year program approved in 1971. The current grant is for $54,338.

The pending request is for $54,625, less than originally estimated.

Documents Submitted (attached)

1. Application dated January 26, 1973.

2. Progress report, July 1, 1972 - January 1, 1973.

Comments

Dr. Dawber has promised to send manuscripts of relevant papers now in press, as listed under Item 11, page 2 of the application.

F.W.N.

FWN:wg
Encls.

1003539385

Source: https://www.industrydocuments.ucsf.edu/docs/ynl0006

CTR Grant #826-R1

Progress Report No. 2

Thomas R. Dawber, M.D., M.P.H. - Principal Investigator
Boston University School of Medicine
80 East Concord Street
Boston, Massachusetts 02118

Dates covered:
July 1, 1972 -
January 1, 1973

Title of Grant: SMOKING CLASSES, RISK FACTORS AND CARDIOVASCULAR DISEASE

The examination of the Framingham Study population and the evaluation of deaths and illness in the population has proceeded according to schedule. Four thousand one hundred ninety-three subjects still remained alive at the time the study was begun in September 1971. The schedule called for examination of 2,513 of these subjects through October 1972. Of this number 1,934 completed their examination. The 579 not yet examined are accounted for as follows:

Exam scheduled	53
Died before exam	56
Incapacitated (but living in Framingham)	51
Not living in area	102
Refused	104
No response to contact by mail	96
Address unknown	65
No information available in current file	52

An additional 106 of the remaining 1,580 scheduled for examination by September 1973 have also been examined slightly ahead of schedule. We therefore have good reason to anticipate success in re-evaluating the entire population by September 1973.

Some problems have developed relative to the availability of past data on the subjects in the study. The National Heart and Lung Institute

10035393386

Source: https://www.industrydocuments.ucsf.edu/tobacco/

has been unwilling to release the data which have been processed by them until a re-examination of the population has been completed. This has made it necessary for us to prepare data in our possession for processing. This procedure is currently under way and when completed will provide us with our own set of information on the previous examinations. From these data and our current examination findings we will be able to carry out the necessary analyses.

Meanwhile we have been reviewing records to collect additional data regarding the age of onset of myocardial infarction and other manifestations of coronary heart disease in smokers and non-smokers. This calls for a comparison of individuals at the same age at entry into the study. The principal investigator has been working on this project and has been attempting to solve certain problems involved in making the comparison. It is believed that most of these problems are now solved and that we can proceed to develop a manuscript covering the subject at an early date in 1973. Preliminary observations suggest that the time gap between the age of development of myocardial infarction in cigarette smokers versus non-smokers decreases with age. Continued observations of the population will be necessary to determine whether cigarette smoking merely precipitated a slightly earlier development of this disorder or was a sine qua non. Current data indicate an excess number of cases in the smoking group over what would be expected and thus suggest that in certain individuals cigarette smoking may not be merely an early precipitator of the event. An attempt will be made to determine whether it may be possible on the basis of certain characteristics to determine the latter group.

1003539387

Source: http://www.tobaccodocuments.ucsf.edu/library.html

The records on pipe and cigar smokers are being reviewed to recode the various smoking categories included in this combined group. The objective is to obtain a group of pure cigar smokers and pure pipe smokers and to determine their outcome. If there are insufficient numbers of subjects in the sub-groups, an attempt will be made to combine our findings with those of one or more other similar studies.

A study of alcohol consumption and coronary heart disease has been carried out and a preliminary manuscript prepared. The findings are such that it appears desirable to await the completion of the current examinations to obtain a twenty-year follow up.

Plans will be made prior to July 1, 1973, for a second examination of the subjects beginning in September 1973. This data will mark the completion of twenty years' observation of the entire cohort. We plan to make a number of reports based on the completion of the twenty years of observation originally planned by the National Heart Institute.

10035938

Source: https://www.industrydocuments.ucsf.edu/docs/yyh0000

Comm.
Dr. Jacobson
Dr. Loosli
Dr. Sommers

#826R2
#826R1 - 7/1/72-6/30/73
#826 - 7/1/71-6/30/72

EPIDEMIOLOGY

THE COUNCIL FOR TOBACCO RESEARCH–U.S.A., INC.

110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

JAN 3 0 1973

Application For Renewal of Research Grant

(Use extra pages as needed)

First Renewal ☐ Second Renewal ☒ Date: Jan. 26, 1973

JAN 3 0 1973

1. Principal Investigator (give title and degrees):

Thomas R. Dawber, M.D., M.P.H. — Principal Investigator
H. Emerson Thomas, Jr., M.D. — Co-Investigator
Carl C. Seltzer, Ph.D. — Co-Investigator

2. Institution & address:

Boston University School of Medicine
80 East Concord Street
Boston, Mass. 02118

1003539389

3. Department(s) where research will be done or collaboration provided:

Department of Medicine
Evans Department of Medical Research

4. Short title of study:

Smoking Classes, Risk Factors and Cardiovascular Disease

5. Proposed renewal date: July 1, 1973

6. How results to date have changed earlier specific research aims: The research aims of this study are essentially unchanged. They include the study of the effect of tobacco smoking on the development of certain diseases. Originally the Framingham Study was set up to investigate the relation of certain constitutional and environmental factors to the development of coronary heart disease. This intent continues but now also involves the investigation of stroke and peripheral vascular disease.

In addition, a clinic has been organized to study the development of cataract, glaucoma, retinal degeneration and diabetic retinopathy. This will be open on February 1, 1973. Included in this investigation will be the effect of tobacco usage habits on these disorders. The conduct of this study is dependent on our ability to bring in the population for study which has been partially supported by the C.T.R. grant (i.e. our success in bringing in the population for study has made it possible to enlarge the scope of the study).

7. How results to date have changed earlier working hypothesis:

No essential change

Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

8. Any additional facilities now required? Describe briefly:

This study has been carried out using facilities currently under contract to the National Heart and Lung Institute. The crowding and inefficiency resulting from having our employees working in cramped quarters has made it necessary that we obtain additional space for the use of the Boston University Medical Center - Framingham Study workers. Accordingly, we have rented approximately 800 square feet of space across the street from the Framingham Union Hospital to be occupied by the principal investigator and certain members of the staff. This space is adjacent to the BUMC - Framingham Study Eye Clinic and will make possible a more efficient operation in continuing all phases of the study.

9. Any changes in personnel? Append biographical sketches of new key professional personnel:

No new personnel have been added

1003539390

10. Append outline of experimental protocol for ensuing year.

11. List publications or papers in press resulting from this or closely related work. (append reprints or manuscripts not previously sent).

a.) Kannel, W. B. and Dawber, T. R.: Atherosclerosis as a pediatric problem. J. Pediat. 80 (#4 - part 1) p. 544-554, April 1972.

b.) Kannel, W. B. and Dawber, T. R.: Epidemiology of coronary disease: Contributors to coronary risk implications for prevention and public health: The Framingham Study. Heart and Lung I (#6), p. 797-810, Nov.-Dec. 1972.

c.) Dawber, T. R., Thomas, H. E., and McNamara, P. M.: Vasculography in the detection of coronary heart disease. To be published in April 1973 in Angiology.

d.) Wolf, P. A., Kannel, W. B., McNamara, P. M. and Gordon T.: The role of impaired cardiac function in atherothrombotic brain infarction: The Framingham Study. Am. J. Pub. Health 63(#1), p. 52-58, Jan. 1973.

e.) Kannel, W. B. and Dawber, T. R.: Hypertensive cardiovascular disease: The Framingham Study. for Hahnemann Symposium -- to appear in Hypertension: Mechanisms and Management.

f.) Dawber, T. R. and Kannel, W. B.: Current status of coronary prevention: Lessons from the Framingham Study. To be published in Preventive Medicine.

12. Summary progress report (append in standard form as separate document, unless recently submitted).

Source: https://www.industrydocuments.ucsf.edu/docs/yjyb0000

10. Research Protocol

The Framingham Study population has been under observation since 1949 with biennial examinations of the subjects to determine the development of certain atherosclerotic diseases, i.e. coronary heart disease, cerebrovascular disease and peripheral arterial disease. In addition, recording has been made of numerous host and environmental factors which may affect the development of these disorders. Among these characteristics are the smoking habits of the population. At the present time the study is being continued by the re-examination of the entire remaining population of over four thousand subjects between September 1, 1971, and September 1, 1973. The Progress Report indicates the status of this study.

During the coming year we plan to:

1. Continue examination of remaining subjects to complete Exam 12 by September 1, 1973

2. Begin re-examination on Exam 13 in September 1973. This calls for:

   (a) preparation of forms for Exam 13 -- based on decisions regarding the collection of new data

   (b) revision of examination procedures

   (c) provision of adequate space for re-examination

   (d) collection and processing of data

It has been necessary to review the records and retabulate the data to permit comparison of subjects entering the study at a given age. Comparison is being made between cigarette smokers and non-smokers in terms of the age of myocardial infarction, sudden death, coronary insufficiency, and angina pectoris. Observations of differences in the age of development of these manifestations are made and estimates of the apparent differences between the smokers and non-smokers derived.

3. Continuation of data processing for Exam 12 and analysis thereof.

1003539391

Source: https://www.industrydocuments.ucsf.edu/docs/xyhd0000

13. Budget for the coming year:

A. Salaries (give names or state "to be recruited")	% time	Amount
Professional (give % time of investigator(s) even if no salary requested)		
Thomas R. Dawber, M. D., M. P. H.	40%	$12,000
H. Emerson Thomas, Jr., M. D.	25%	6,000
Carl C. Seltzer, Ph. D.	20%	5,000
Technical		
Clerk typist	100%	8,250
Statistical clerk	100%	8,250
	Sub-Total for A	39,500
B. Consumable supplies (by major categories)		
Record forms		500
Office supplies		500
	Sub-Total for B	1,000
C. Other expenses (itemize)		
Travel		1,500
Data processing		4,000
Space (40% of cost)		1,500
	Sub-Total for C	7,000
	Running Total of A + B + C	47,500
D. Permanent equipment (itemize)		
None	Sub-Total for D	--
E. Indirect costs (15% of A+B+C)	E	7,125
	Total request	54,625

10035393392

Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

14. Other sources of financial support:

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
The Epidemiology of Stroke	NIND - #NS09695-02	65,194	May 1, 1972 - April 30, 1973
Private funds supplied by Boston University (BUMC-Framingham Study Fund)		50,000	July 1, 1972 - June 30, 1973

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Continuation of above			

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Checks payable to

Boston University

Mailing address for check:

Boston University School of Medicine
80 East Concord Street
Boston, Mass. 02118

Principal investigator

Typed Name Thomas R. Dawber, M.D., M.P.H.

Signature Thomas R Dawber Date 1/26/73

Telephone (617) 262-1400 6524
Area Code Number Extension

Responsible officer of institution

Typed Name Robert N. Jordan

Title Assistant Treasurer - Boston University

Signature Robert N. Jordan Date 1/26/73

Telephone (617) 262-4200 6101
Area Code Number Extension

10035393

Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

CTR Grant #826-R1 Progress Report No. 2

Thomas R. Dawber, M.D., M.P.H. - Principal Investigator
Boston University School of Medicine
80 East Concord Street
Boston, Massachusetts 02118

Dates covered:
July 1, 1972 -
January 1, 1973

Title of Grant: SMOKING CLASSES, RISK FACTORS AND CARDIOVASCULAR DISEASE

The examination of the Framingham Study population and the evaluation of deaths and illness in the population has proceeded according to schedule. Four thousand one hundred ninety-three subjects still remained alive at the time the study was begun in September 1971. The schedule called for examination of 2,513 of these subjects through October 1972. Of this number 1,934 completed their examination. The 579 not yet examined are accounted for as follows:

Exam scheduled	53
Died before exam	56
Incapacitated (but living in Framingham)	51
Not living in area	102
Refused	104
No response to contact by mail	96
Address unknown	65
No information available in current file	52

An additional 106 of the remaining 1,580 scheduled for examination by September 1973 have also been examined slightly ahead of schedule. We therefore have good reason to anticipate success in re-evaluating the entire population by September 1973.

Some problems have developed relative to the availability of past data on the subjects in the study. The National Heart and Lung Institute

1003539394

Source: https://www.industrydocuments.ucsf.edu/docs/yjgl0000

has been unwilling to release the data which have been processed by them until a re-examination of the population has been completed. This has made it necessary for us to prepare data in our possession for processing. This procedure is currently under way and when completed will provide us with our own set of information on the previous examinations. From these data and our current examination findings we will be able to carry out the necessary analyses.

Meanwhile we have been reviewing records to collect additional data regarding the age of onset of myocardial infarction and other manifestations of coronary heart disease in smokers and non-smokers. This calls for a comparison of individuals at the same age at entry into the study. The principal investigator has been working on this project and has been attempting to solve certain problems involved in making the comparison. It is believed that most of these problems are now solved and that we can proceed to develop a manuscript covering the subject at an early date in 1973. Preliminary observations suggest that the time gap between the age of development of myocardial infarction in cigarette smokers versus non-smokers decreases with age. Continued observations of the population will be necessary to determine whether cigarette smoking merely precipitated a slightly earlier development of this disorder or was a sine qua non. Current data indicate an excess number of cases in the smoking group over what would be expected and thus suggest that in certain individuals cigarette smoking may not be merely an early precipitator of the event. An attempt will be made to determine whether it may be possible on the basis of certain characteristics to determine the latter group.

1003539395

Source: https://www.industrydocuments.ucsf.edu/docs/

The records on pipe and cigar smokers are being reviewed to recode the various smoking categories included in this combined group. The objective is to obtain a group of pure cigar smokers and pure pipe smokers and to determine their outcome. If there are insufficient numbers of subjects in the sub-groups, an attempt will be made to combine our findings with those of one or more other similar studies.

A study of alcohol consumption and coronary heart disease has been carried out and a preliminary manuscript prepared. The findings are such that it appears desirable to await the completion of the current examinations to obtain a twenty-year follow up.

Plans will be made prior to July 1, 1973, for a second examination of the subjects beginning in September 1973. This data will mark the completion of twenty years' observation of the entire cohort. We plan to make a number of reports based on the completion of the twenty years of observation originally planned by the National Heart Institute.

100539396

Source: https://www.industrydocuments.ucsf.edu/docs/[illegible]

#623BR2 ESSMAN

10035393397

Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

THE COUNCIL FOR TOBACCO RESEARCH – U.S.A., INC.

P-3

January 16, 1973

Grant Application No. 623BR2

TO: The Committee comprising Drs. Andervont, Cattell and Meier

SUBJECT: Walter B. Essman, M.D., Ph.D., Professor
Queens College of the City University of New York
Second Renewal Application No. 623BR2
"Studies of Nicotine Action Upon Memory Consolidation"

History

CTR has supported this investigator since 1968.

Application No. 623BR2 requests $21,579.00 for the third and final year of a three year program approved by SAB late in 1970 (This requestis slightly less than originally estimated for the third year of this program).

Documents Submitted (attached)

1. Application dated January 9, 1973.

2. Progress Report No. 6, February 1, 1972 - January 15, 1973.

3. Reprints or manuscript of the three papers cited on Page 5 of the Progress Report.

Comment

Unexpended funds of the previous Grant No. 623BR1, will be used to keep this investigation viable from February 1, 1973 through March 31, 1973.

F.W.N.

FWN/vr
Encl.

1003539398

Source: https://www.industrydocuments.ucsf.edu/docs/[illegible]

Grant #623 BR-2
#623 BR-1
#623
Act: 2/1/71
#623A
Act. 2/1/70
#623
Act: 2/1/68
Ren. 2/1/69

PHARMACOLOGY

Comm.
Dr. Andervont
Dr. Cattell
Dr. Meier

THE COUNCIL FOR TOBACCO RESEARCH–U.S.A., INC.
110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

JAN 15 1973

Application For Renewal of Research Grant
(Use extra pages as needed)

First Renewal ☑ Second Renewal ☒ Date: Jan. 9, 1973

1. Principal Investigator (give title and degrees):

Walter B. Essman, M.D., Ph.D., Professor

2. Institution & address:

Queens College of the City University of New York
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Flushing, New York 11367

AND

Research Foundation of the City University of New York
1411 Broadway
New York, New York 10018

3. Department(s) where research will be done or collaboration provided:

Departments of Psychology and Biochemistry
Queens College of the City University of New York

4. Short title of study:

Studies of Nicotine Action Upon Memory Consolidation

5. Proposed renewal date: April 1, 1973

6. How results to date have changed earlier specific research aims:

Those results obtained to data have not changed earlier specific research aims.

7. How results to date have changed earlier working hypothesis:

There have been no essential changes in earlier working hypotheses.

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8. Any additional facilities now required? Describe briefly:

No additional facilities are required.

9. Any changes in personnel? Append biographical sketches of new key professional personnel:

No personnel changes are indicated and no additional professional personnel additions are contemplated.

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10. Append outline of experimental protocol for ensuing year. See attached protocol.

11. List publications or papers in press resulting from this or closely related work. (append reprints or manuscripts not previously sent).

Alterations in the behavioral and biochemical effects of electroconvulsive shock with nicotine. Psychon. Sci., 1968, 12, 107-108 (with M.I. Steinberg and M.I. Golod).

Alterations in brain serotonin metabolism mediating enhanced memory consolidation. In: Cerletti, A., & Bove, F.J. (Eds.) The Present Status of Psychotropic Drugs. Amsterdam: Excerpta Medica, 1969, Pp. 305-306.

Central nervous system metabolism, drug effects, and higher functions. In: Smith, W.L. (Ed.) Drugs and Cerebral Function. Springfield: Chas. Thomas, 1970, Pp. 151-175.

The role of biogenic amines in memory consolidation. In: Adám, G. (Ed.) The Biology of Memory. Budapest: Akademiai Kiado Publ., 1970, Pp. 213-238.

Metabolic and behavioral consequences of nicotine. In: Smith, W.L. (Ed.) Cerebral Function Development and Drug Action. Springfield, Ill., Chas. Thomas, 1972, Pp. 273-287.

(publications continued on attached sheet)

12. Summary progress report (append in standard form as separate document, unless recently submitted).

Source: https://www.industrydocuments.ucsf.edu/docs/ytyl0000

Drugs affecting facilitation of learning and memory. In: Rubin, A.L. (Ed.) Search for New Drugs. N.Y.: Dekker, 1972.

Drug effects and learning and memory processes. In: Garattini, S., and Shore, P. (Eds.) Advances in Pharmacology and Chemotherapy. N.Y.: Academic Press, 1971b, Pp. 241-330.

Cholinergic mechanisms and avoidance behavior acquisition: effects of nicotine in mice. Psychol. Rep., 1971, 29, 987-993 (with S.G. Essman).

Changes in cholinergic activity and avoidance behavior by nicotine in differentially housed mice. Int. J. Neurosci., 1971, 2, 199-206.

Nicotine-related neurochemical changes: some implications for motivational mechanisms and differences. In: Dunn, W.J., Jr. (Ed.) Motivation in Cigarette Smoking, 1972 (In Press).

Neurochemical modulation of experimentally induced retrograde amnesia. Confinia Neurol., 1973 (In Press).

Age dependent effects of 5-hydroxytryptamine upon memory consolidation and protein synthesis. Physiol. Behav., 1973 (In Press).

Effects of ECS and intracranial 5-HT on cerebral protein synthesis. Paper presented at Fourth Annual Winter Conference on Brain Research, Vale, Colorado, Jan. 1972 (with E. Heldman).

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13. Budget for the coming year:

A. Salaries (give names or state "to be recruited")	% time	Amount
Professional (give % time of investigator(s) even if no salary requested)		
Technical		
Technical Assistant (to be selected)	100%	8,500.00
Grant Technician (Sheryl Sherman)	50%	4,340.00
Fringe Benefits		2,054.00
	Sub-Total for A	14,894.00
B. Consumable supplies (by major categories)		
Animal Purchases		1,100.00
Animal food, bedding, and cleaning supplies		600.00
Chemical and glassware		770.00
Disposable cages		300.00
	Sub-Total for B	2,770.00
C. Other expenses (itemize)		
Travel: International Symposium on Serotonin, Sassari, Sardinia, May, 1973 Federation of Societies for Experimental Biology, Atlantic City, New Jersey, April, 1973 Winter Conference on Brain Research, Vale, Colorado, Jan., 1974	Sub-Total for C	1,100.00
	Running Total of A + B + C	18,764.00
D. Permanent equipment (itemize)		
None		
	Sub-Total for D	
E. Indirect costs (15% of A+B+C)	E	2,815.00
	Total request	21,579.00

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14. Other sources of financial support:
List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Metabolic Responses to Stress-Tobacco Smoke Interactions	C.T.R.	$40,000.00	10/1/72 - 9/30/73

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Checks payable to
Research Foundation of C.U.N.Y.

Mailing address for check:
1411 Broadway
New York, New York 10018

Principal investigator
Typed Name Walter B. Essman
Signature Walter B Essman Date 1/10/73
Telephone 212 762-5949
Area Code Number Extension

Responsible officer of institution
Typed Name Nathaniel H. Siegel - Vice President
Title Hannah Petzenbaum - Research Foundation Queens College
Signature _____ Date _____
Telephone _____
Area Code Number Extension

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10. Brief Description of Objectives or Specific Aims:

The overall goal of this project is to relate several of the effects of nicotine treatment in experimental animals to the process of memory consolidation. This relationship will be explored in several experiments designed to study the metabolic effects of nicotine treatment under several conditions, and to relate these effects to behavioral paradigms, within which memory consolidation can be objectively defined. Previous results have substantially supported the hypothesis that the administration of nicotine sulfate, at critical times prior to training for acquisition of a conditioned response can, under appropriate conditions, facilitate memory consolidation through the attenuation of an experimentally-induced amnesia effected by post-conditioning electroconvulsive shock.

The time course of nicotine effects in relation to both memory consolidation and central metabolic events, which parallel experimentally-induced disruption of such consolidation, has been defined in prior studies, and several metabolites of nicotine have been specified as possibly accounting for the facilitation of memory consolidation. The interaction between nicotine metabolites and the amnesic event, electroconvulsive shock, has been considered in a series of studies which have indicated that the regional metabolism of serotonin (5-hydroxytryptamine) in the brain can be altered by nicotine, its metabolites, and the interaction of these with the amnesic properties of post-conditioning electroconvulsive shock.

The major objective of continued investigation of nicotine action and the memory consolidation process concerns four specific areas wherein this relationship will be intensively investigated; these areas of further investigation represent those which have previously been outlined for the full three years of grant support request, for which the first year was approved and is currently active.

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The two aspects of the initially proposed areas that have been extensively explored within the currently active grant period bear upon the relationship between nicotine and its metabolites to the amnesic properties of post-conditioning agents or events as a means of assessing parameters relevant to the memory consolidation process. Further exploration will concern: (1) the effects of nicotine and several of its active metabolites upon memory consolidation as a function of development; (2) identification of the regional uptake and distribution of nicotine metabolites in the brain following administration, and thereby to relate uptake and distribution to cellular and subcellular effects; (3)the temporal relationship between central uptake and distribution and alterations in cerebral amine and metabolism; and (4) specific cellular effects of nicotine and its metabolites as these effects relate to the memory consolidation process and its disruption by specific amnesic agents or events.

Progress in the current area of investigation has been summarized in the appended reports and specifically detailed in the included reprints and preprints of published papers. These areas of completed work and work in progress have concerned the effects of chronic administration of nicotine in mice where such effects have been behaviorally evaluated utilizing both post-and-active avoidance conditioning procedures whereas the retention of passive avoidance behavior as currently facilitated through such chronic treatment, the retention of active avoidance behavior appears to be selectively impaired by such chronic treatment, depending upon endogenous conditions conferred on the basis of differential housing. This line of investigation has yielded an extremely useful model for memory retardation in avoidance conditioning, where such memory pathology can be appreciably attenuated through the chronic administration of nicotine. A wide aspect of the proposed work which, again has seen considerable pro-

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gress made, concerns the cellular effects of nicotine, particularly as these are concerned with cholinergic transmission at the synapse. Extension studies utilizing subcellular fractionation techniques have indicated that in specific regions of the mouse cerebral cortex, the acetylcholine content of synaptic vesicles represent thirty per cent of the bound acetylcholine in cerebral cortex; this contrasts with eleven per cent vesicular acetylcholine in the cerebral cortex of control animals. Furthermore, a time course study relating cholinergic changes to the amnesic effect of post-training electroconvulsive shock indicated that the optimal intervals for ECS induced amnesia, there was a twenty per cent decrement in vesicular acetylcholine levels. This observation again contrasts with the finding that animals treated with nicotine and then given electroconvulsive shock showed a sixty-four per cent increase in vesicular acetylcholine content. These findings, similar to previous observations concerned with serotonogenic changes brought about by nicotine and its metabolites, point to important basic cellular effects which underly basic mechanisms for those cognitive processes affected by nicotine. A further aspect of the cellular specificity of those effects brought about by nicotine and its metabolites has also been in progress over the past year and continues to yield results. These studies have utilized cellular separation techniques which allow a distinction to be made between a neuron-enriched and a flia-enriched fractionation from several representative areas of the mouse brain. Cellular effects of nicotine and several of its metabolites have been studied within this context insofar as time course, regional specificity of effect, and the nature of the changes in biogenic amines asfar as their cellular specificity.

It is anticipated that this work which will include data based on study-state kinetic estimates of cellular timeover for biogenic amines will be completed within approximately eight months and will therefore constitute an important basic step upon which further studies may then be elaborated.

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7. Brief Statement of Working Hypothesis:

The administration of nicotine and several of its centrally active metabolites can, within the appropriate temporal and dosage parameters, alter the time course and efficacy of the memory consolidation process through effects initiated by cellular and regional alterations in brain amine metabolism. The process of memory consolidation, as facilitated by nicotine treatment (chronic, post-trial, or as a function of age), will be modified through alterations in centrally active biogenic amines, which may underlie the basic of experimentally induced retrograde amnesia,and through such modifications, a reduction in amnesia may be viewed as facilitation of memory consolidation.

8. Details of Experimental Design and Procedures:

The behavioral portion of all the experiments to be carried out will employ the single trial conditioning procedure, previously described in our protocols for establishing a stable avoidance response in one trial. This response, which is stable over time as well as within experimental conditions, has been successfully utilized to assess the amensia effect of electroconvulsive shock and other agents or events which, when presented in close temporal proximity with the acquisition trial experience, result in a reduction in the incidence of retention, as measured 24 hours later; amnesia and/or retention are assessed by measures of response latency so that criterion avoidance is defined on the basis of latencies in excess of five standard deviations of the mean latency exhibited on the training trial and an absence of retention, or retrograde amnesia, is defined as a response latency on the testing trial that is equivalent to or within one standard deviation of the mean response latency shown on the training trial. This procedure has been successfully utilized in our laboratory with over 20,000 mice during the nine to ten years within which the technique was originally devised by this investigation. A more detailed description of procedure, parameters covering acquisition and

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retention, and some of the applications thereof, may be found in several publications (Essman & Alpern, 1965; Essman, 1968; Essman and Essman, 1969).

Animals utilized in all of the proposed studies will be CF-1 strain mice, (Mus Musculus), to be obtained from a commercial vendor (Carworth Farms, Inc., New City, New York), at weaning (21 days) and following adaptation to conditions of laboratory use (10/cage), will be utilized as experimental subjects at approximately 30 days of age or, in the case of the developmental studies proposed, animals will be bred in the laboratory specifically for purposes of obtaining precise age controls. The specific experiments to be conducted within the time specified for the proposed research will concern:

(1) the effects of nicotine and its active metabolites upon memory consolidation during early development;

(2) identification of the regional uptake and distribution of nicotine metabolites in the brain following administration;

(3) the temporal relationship between central uptake of nicotine and its metabolites and the distribution and alterations in biogenic amines;

(3) specific cellular effects of nicotine and its metabolites as related to memory consolidation;

9. Physical Facilities Available:

Two large air-conditioned laboratories and adjoining animal housing facilities ara available and currently in use by the investigator; the laboratories are completely equipped with all equipent necessary for the biochemical procedures required, with the exception of those equipment items requested in the budget. Additional studies will be concerned with the functional role of differential housing as a model for the induction of deficits in memory consolidation and the degree to which both acute and chronic administration of nicotine can alter these deficits. More specific, these experiments will concern a comparison of memory consolidation upon mice housed either in isolation or in groups following weaning

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for various durations, the duration governing the apparent extent to which the memory consolidation deficit is in evidence. Studies in this are will therefore be directed toward evaluation of:

(a) Effects of acute pre-training nicotine administration;

(b) Effect of chronic pre-training nicotine administration;

(c) Interaction of acute pre-training nicotine treatment with electroconvulsive shock as an amesic agent;

(d) Interaction with post-training, post-ECS acute nicotine administration;

(e) Effects of pre-training intracranial nicotine administration;

(f) Effects of post-training intracranial nicotine administration;

In all cases the experiments proposed within this area of the investigation are employing the differentially housed mice indicated, with differring durations of isolation governing the degree to which the memory consolidation deficit occurs.

In the first series of experiments, the effects of chronic nicotine and its metabolites will be investigated in order to assess and compare chronicity of treatment with results already obtained on acute administration. Mice, beginning at approximately 30 days of age, will be given single daily injections of nicotine sulfate within a dose range of 0.25 mg/kg to 2.00 mg/kg, i.p. An equivalent volume of 0.9% saline will be given control animals. Compared with a single acute administration over the dose range indicated, animals will be given injections for 3 days, 7 days, and 21 days, with a single training trial given one hour following the final chronic administration. For each of these conditions, asingle post-training electroconvulsive shock will be given either immediately, 10 minutes, 20 minutes, or 60 minutes following the training trial. A testing trial will be given 24 hours following training, and the incidence of conditioned response retention will be measured. Data emerging from these experiments will allow for statements

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regarding the temporal gradient foe ECS-induced retrograde amnesia as a function of both nicotine sulfate dosage and the duration of nicotine sulfate treatment at a given dosage prior to training. Inasmuch as preliminary data has clearly indicated that the temporal gradient for ECS-induced retrograde amnesia falls within one hour, the proposed intervals for post-training ECS will allow for statements regarding either a contraction of the temporal gradient (i.e. , a reduction of the post-training time interval within ECS results in retrograde amnesia), or an expansion of the gradient (i.e., an extension of the time following training, within which ECS will produce a retrograde amnesia). Since previous data have clearly indicated that the temporal gradient for retrograde amnesia is appreciably shortened as a redult of pretreatment with a single dose of nicotine sulfate, the extent to which, within the present study, these alterations hold as a result of chronic treatment, will be determined. Paralleling the present study, groups of animals, given nicotine sulfate, followed by acquisition training with sham-ECS, will serve as both controls for the effect of ECS as well as provide a baseline from which the effects of chronic nicotine treatment upon acquisition of the conditioned response may be assessed.

Once the conditions under which appropriate dosage and effects of prolonged nicotine treatment are determined as an outcome of the present experiments, the selected group of related compounds and metabolites of nicotine will be utilized in similar chronic studies. These will include nornicotine, (-) cotinine, 3, pyridylacetic acid, myosmine, and anabasine, and will be given under the same conditions of chronic treatment in doses ranging from 0.10 mg/kg to 0.50 mg/kg.

Training, ECS, and sham-ECS treatment and post-training retention trials, as described for the previous experiments, will be given, and the degree to which the temporal gradient for retrograde amnesia is affected by these compounds will

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be assessed in a manner similar to that described above. These experiments should provide data which will allow for statements regarding the effects of chronic nicotine and metabolite treatment upon memory consolidation. The experimental literature contains virtually no studies regarding the behavioral effects of chronic nicotine treatment, and any behavioral toxicity associated with chronicity may be determined from these studies; more specifically, should such findings emerge, these may then be more clearly identified with one of the specific metabolites.

The effects of post-conditioning drug treatment upon memory consolidation has been explored by several investigators with somewhat controversial and discrepancy results emerging. The general premise underlying such studies is that the process of memory consolidation, which is usually initiated by a learning experience, lasts for a period of time following such an experience, within which the process may possibly be enhanced by appropriate drug treatment. If this interval has superimposed upon it a disruptive agent, such as electroshock, a high incidence of retrograde amnesia for the experience results. If the training-ECS sequence is followed by central changes, associated with drug treatment, the disruptive effect of ECS and, possibly, the rate of consolidation might be expected to be altered, providing that the parameters for such a sequence are appropriate. The major purposes of the second series of experiments will be to investigate the results of administered effective doses of nicotine (1 mg/kg) and its metabolites (0.25 mg/kg) upon the amnesic effect of electroshock, where such treatment is given following ECS. The training-ECS interval will be adjusted to coincide with those post-training times within which, based upon previous data, ECS is effective in producing retrograde amnesia. Nicotine sulfate and the above-named metabolites will be given either immediately, 1 minute, 5 minutes, or 10 minutes following ECS administration,

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and a testing trial to measure the incidence of retrograde amnesia and/or conditioned response retention will be given 24 hours following training.

The critical parameters in this series of studies, therefore will be: (a) the training-ECS-drug treatment interval, and (b) the interaction of these intervals with the training-ECS interval. Control groups, receiving physiological saline, paralleling the above described conditions, and drug-treated controls, given sham-ECS, will be utilized to evaluate the significance of emerging results.

The major point toward which this series of experiments is directed is whether the memory consolidation interval is sufficiently labile to permit the interposition of any post-amnesic treatment nicotine effects.

A further series of experiments will concern the relationship between nicotine and several of its active metabolites upon the memory consolidation process as a function of development. Previous data, summarized in the supporting data section of this application, have indicated that the CF-1 strain mouse, at 17 days of age, is highly resistant to the amnesic effect of electroconvulsive shock, as compared with younger or older mice of the same strain, and that mice at 15 and 16 days of age show a reduced incidence of ECS-induced retrograde amnesia, as compared to mice of 20 days, or more, of age. A developmental parllel to these behavioral findings appears to reside in levels of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid, and also in the turnover of the former in the brain.

Conditions which maximize resistance to retrograde amnesia, induced by ECS, appear to be developmentally dependent, and it is within this context that the effects of nicotine and several of its metabolites will be investigated. The basic hypothesis underlying this series of studies is that the facilitation of memory consolidation by nicotine and its active metabolites will be age-dependent insofar as age-dependent differences in memory consolidation are present. It would therefore seem reasonable to assume that nicotine and its active metabolites will be

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more effective in altering memory consolidation at those ages where the amnesic effect of electroshock is maximal. Groups of mice, ranging in age from 15 to 30 days, will be treated with either nicotine sulfate (1.0 mg/kg) or one of its active metabolites (0.25 mg/kg) 45 to 60 minutes prior to the administration of a single training trial, as previously described, followed at intervals of either 0, 10, 20, or 60 minutes by a single transcorneal ECS. A testing trial, given 24 hours following training, will assess the extent to which drug treatment was effective in attenuating or antagonizing the amnesic effect of electroshock.

Control groups, given physiological saline under each of the experimental conditions specified, and drug-treated animals, given sham-ECS, will be utilized as controls. The differential alterations in brain 5-hydroxytryptamine level and metabolism during early development represents an ideal system within which nicotine and its metabolites, as agents which, in themselves, alter relatively stable levels of 5-HT metabolism, may be investigated. Within this context, there resides the important question as to whether nicotine and its metabolites, in producing predictable alterations in amine metabolism, will do so reversibly during early development, or whether these effects during early development result in changes, the magnitude or duration of which may be considerably discrepant from that observed in the adult animal. For these reasons, it will be a further purpose of this investigation to study the changes produced by nicotine and its active metabolites upon brain 5-HT levels and metabolism during early development. These procedures will essentially parallel the behavioral portion of the study, as outlined above, and will follow through the use of techniques previously described.

Through the use of spectrofluorometric procedures, developed in this laboratory, it should be possible to characterize nicotine and its active metabolites and, based upon such characterization, to identify regional locus following systemic

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injection. These procedures will basically involve a series of experiments in which, at several times following the systemic injection of nicotine sulfate or active metabolites, mentioned above, mice will be sacrified by cervical dislocation, the whole brain will be removed, transferred immediately to freon, chilled in a jacked of liquid nitrogen, and upon rewarming to -30° C, macrodissection will be carried out for isolation of several areas of the brain, to include: olfactory bulbs, cerebral cortex, mesencephalon, diencephalon, limbic system structures, hypothalamus, and cerebellum. From each of these areas spectrofluorometric determination of previously standardized procedures for estimation of nicotine and its metabolites will be carried out and, in addition, the distribution of centrally active biogenic amines will be concommitantly determined from the same regions. These studies will allow for statements to be made regarding (a) the regional uptake and distribution into the central nervous system of nicotine and its metabolites following central administration, (b) the temporal relationship between dosage and central uptake, and (c) the temporal relationship between central utake, distribution, and changes in the metabolism of biogenic amines. In addition to obtaining data concerned with serotonin, which is the basis of the theoretical model within which nicotine effects and memory consolidation are being investigated in the present studies and those proposed, the availability of regionally specific tissue from mouse brain in the proposed studies also allows for determination of acetylcholine levels and acetylcholinesterase activity. In the latter regard, there has been no published information on the relationship between nicotine uptake and its metabolite distribution in the central nervous system, and cholinergic changes which relate temporally to these conditions.

A final aspect of the proposed work relates to the cellular effects of nicotine

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and its metabolites, as these are related to memory consolidation. This issue is given preliminary support in this application by methodological and empirical findings from our laboratory, which suggest the feasibility of obtaining reasonably purified neuronal and glial fractions from mouse brain and demostrating that in vivo treatment, resulting in retrograde amnesia in mice, exerts a differential effect at the cellular level; i.e., neurons are affected over a differentiate time course than glia following ECS, and the effect in glia is more prolonged than that in neurons. The specific effect to which these findings are in alterations in 5-hydroxyindoleacetic acid following electroconvulsive shock. Through the utilization of the fractionation technique, which involves a discontinuous sucrose-ficoll density gradient centrifugation, a neuronal and glial pool can be separated from myelin and red cells. These fractions have been morphologically verified in both phase-contrast and electron microscopy and their purity has been verified by the relative rations of acetylcholinesterase in the respective fractions. The purpose of this final series of experiments will be to assess the effect of nicotine and its metabolites on cellular levels of biogenic amines, and study the interaction of drug treatment with electrcconvulsive shock upon these cellular levels. Specifically, we may anticipate that cellular changes, which parallel retrograde amnesia produced by ECS, will be blocked, attenuated, or antagonized by nicotine or its active metabolites under these conditions where these behaviorally modify the amensic effect of elctroshock. All of the experimental conditions will be applied in vivo, following which the brains will be removed, prepared, and extracted, as previously described, with the addition of the fractionation procedure for isolation of neuronal and glial pools in several of the areas described. These studies should allow for a considerable degree of useful data to emerge regarding differences in the regional cellular response in the central nervous system to nicotine and its meta-

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bolites. It should be noted that this question has not, in any case, been treated in any of the experimental literature and costitutes what is probably an exceedingly important issue insofar as the central effects of nicotine are concerned.

The additional studies that have been proposed concern (Table 7) the application of a differential housing model for the evaluation of facilitative as well as potentially disruptive effects of nicotine treatment upon memory consolidation can be governed by the duration of isolation housing under which animals post weaning are maintained. The six experimental aspects of this investigation have been outlined in an earlier part of the application and the biochemical assessment of nicotine induced changes are paralleled those procedures previously described.

Should additional results warrant further investigation of cholinergic mechanisms relating either to retrograde amnesia or the interaction of this phenomenon with nicotine treatment, additional studies will be initiated, utilizing subcellular fractionating techniques to evaluate the description of cholinergic effects mediated at the nerve ending which offer implications for the role synaptic processes in nicotine mediated changes in memory consolidation.

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Grant #623BR2

The Council for Tobacco Research - U.S.A. Grant, "Studies of Nicotine Action upon Memory Consolidation."

INTERIM
PROGRESS REPORT NO. 6

Walter B. Essman, M.D., Ph.D.
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Feb. 1, 1972 -
Jan. 15, 1973

STUDIES OF NICOTINE ACTION UPON MEMORY CONSOLIDATION

The research project beyond establishing several of the interrelationships between the behavioral effects of nicotine and several of its biologically active metabolites and certain specific putative neurotransmitter molecules active in brain metabolism has, within the past year, specifically concerned itself with the interaction of these events and experimentally-induced retrograde amnesia. Whereas previous research has fundamentally established a strong basis for changes in brain serotonin metabolism associated with retrograde amnesic events, such as electroconvulsive shock (ECS), in mice and further that such ECS is temporally contiguous with an inhibition of cerebral protein synthesis sufficient to account for the amnesic phenomenon (Essman and Heldman, 1972), several experiments were systematically undertaken in order to further examine the direct role of cerebral serotonin in mediating the amnesic effect. In previous progress reports, as well as published papers, we have indicated that nicotine as well as several of its biologically active metabolites may well antagonize the amnesic effect of ECS by blocking the cerebral changes in serotonin metabolism usually attendant upon ECS treatment. Several experiments have further corroborated this result and supported the hypothesis that not only is the change in serotonin metabolism necessary to experimentally produce a retrograde amnesia, but an inhibition of protein synthesis is also such a requisite.

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Both such changes haje been shown to be attenuated or blocked by nicotine.

Specific experiments have considered the age dependent nature of experimentally-induced amnesia and the extent to which such age dependency includes developmental as well as critical age phenomena dependent upon brain serotonin metabolism. Specifically, subcellular fractionation of the mouse cerebral cortex has revealed that such changes reside within the presynaptic nerve ending (synaptosome). Amnesic effects, as well as effects upon cerebral protein synthesis, appear to be specific to an initial increase in local concentration of serotonin, as supported by experiments within which this amine was micro-injected into the hippocampal region of the brain; retrograde amnesic effects as well as inhibition of protein synthesis was in evidence under such conditions and these effects appeared to be specific to serotonin, inasmuch as analogs thereof or other biogenic amines did not provide for either the amnesic or synthetic effects.

Whereas an age specific phenomenon for susceptibility to retrograde amnesia was previously shown for ECS-induced conditions, it was further shown that intracranial 5-HT treatment not only induced an age dependent amnesia but also a highly correlated age dependent inhibition of protein synthesis.

The indole amine changes in brain as a function of nicotine have been indicated such that a marked increase in brain 5-hydroxytryptamine (5-HT) turnover time results. This effect has been shown to be regionally specific in brain as well as time dependent following nicotine treatment. The changes in the relationship between 5-HT and its metabolite in brain 5-HIAA, has also been shown to be re-

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gionally specific over time.

Investigation of cell specific populations isolated following in vivo treatment with nicotine indicated that increases in 5-HT content were specific to both neurons and glia isolated from different regions of the mouse brain. The magnitude of such increments was regionally specific for brain and also cell specific for such given regions.

The cholinergic effects of nicotine, although well-known, were further investigated on a subcellular level to specifically examine changes in the size of various cholinergic pools in the cerebral cortex. These results have indicated that administration of nicotine leads to a decrease in the acetylcholine content of both bound and vesicular storage pools without any alteration in the concentration of free acetylcholine.

The effects of nicotine were also examined in relation to the differential effect of this compound among environmentally conferred differences in learning ability. These previously published data have indicated that task-specific facilitative effects of nicotine exert different effects both behaviorally and metabolically depending upon the endogenous behavioral and metabolic baseline upon which they are superimposed. Such a model has been derived utilizing differentially housed mice. In animals treated with radioactive nicotine it was found that isolated animals showed a greater brain uptake, for several regions of nicotine, as compared to group housed mice, where the only appreciable uptake occurred in the basal ganglia; the latter change, however, was markedly below the uptake of nicotine shown by isolated animals. Nicotine metabolites, specifically cotinine, were also found

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to be present in considerably greater quantities in several regions of the brain of isolated animals. This finding would be consistent with our previous suggestion that the facilitative effect of nicotine treatment upon the learning ability of behaviorally retarded mice (as result from early isolation housing) may be dependent upon a biologically active metabolite of nicotine, rather than to nicotine per se. A likely candidate for such a metabolite is cotinine, which we have observed to exert potent biochemical and behavioral effects in earlier studies.

In several studies we have also examined the regional differences in cerebral protein synthesis exerted by nicotine at several subcellular sites. In this regard, we have conclusively established that microsomal protein synthesis in mouse cerebral cortex is significantly augmented as a consequence of nicotine treatment, and further that the protein synthesis inhibition which may be environmentally produced by the stress of isolation housing may be completely reversed at the microsomal level by treatment with nicotine. In this respect preliminary studies of mitochondrial proteins from mouse cerebral cortex have indicated temporal qualitative and quantitative differences in the effects of nicotine. It is our purpose to further examine these changes and characterize them with respect to some of the metabolites of this compound.

In a final series of studies, the progress of which may be indicated at this point, it was shown that an experimentally-induced retrograde amnesia effected in mice with electroconvulsive shock could be antagonized or potentiated depending upon the interval of time between nicotine treatment and the post-conditioning amnesic event. In this

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regard, it was shown that conditioned response retention was reduced below control levels if nicotine treatment was given 15 minutes prior to ECS, whereas if such treatment was effected 45 minutes prior to treatment approximately 80% of the animals showed antagonism toward the amnesic effect. These results again are consistent with our earlier findings and provide further support for the general working hypothesis that the role of nicotine in memory consolidation is to provide temporally related facilitative effects as are dependent upon aminergic and protein synthetic interactions in the central nervous system.

Publications Resulting from This Project

1. Essman, W.B. & Essman, S.G. Biphasic effects of nicotine upon ECS-induced retrograde amnesia in mice. Psych. Rep., 1973 (In Press).

2. Essman, W.B. Neuromolecular modulation of experimentally-induced retrograde amnesia. Confinia Neurol., 1973 (In Press).

3. Essman, W.B. Nicotine-related neurochemical changes: some implications for motivational mechanisms and differences. In: Dunn, W.J., Jr. (Ed.) Motivation in Cigarette Smoking, 1972 (In Press).

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#24R-17 LARSON

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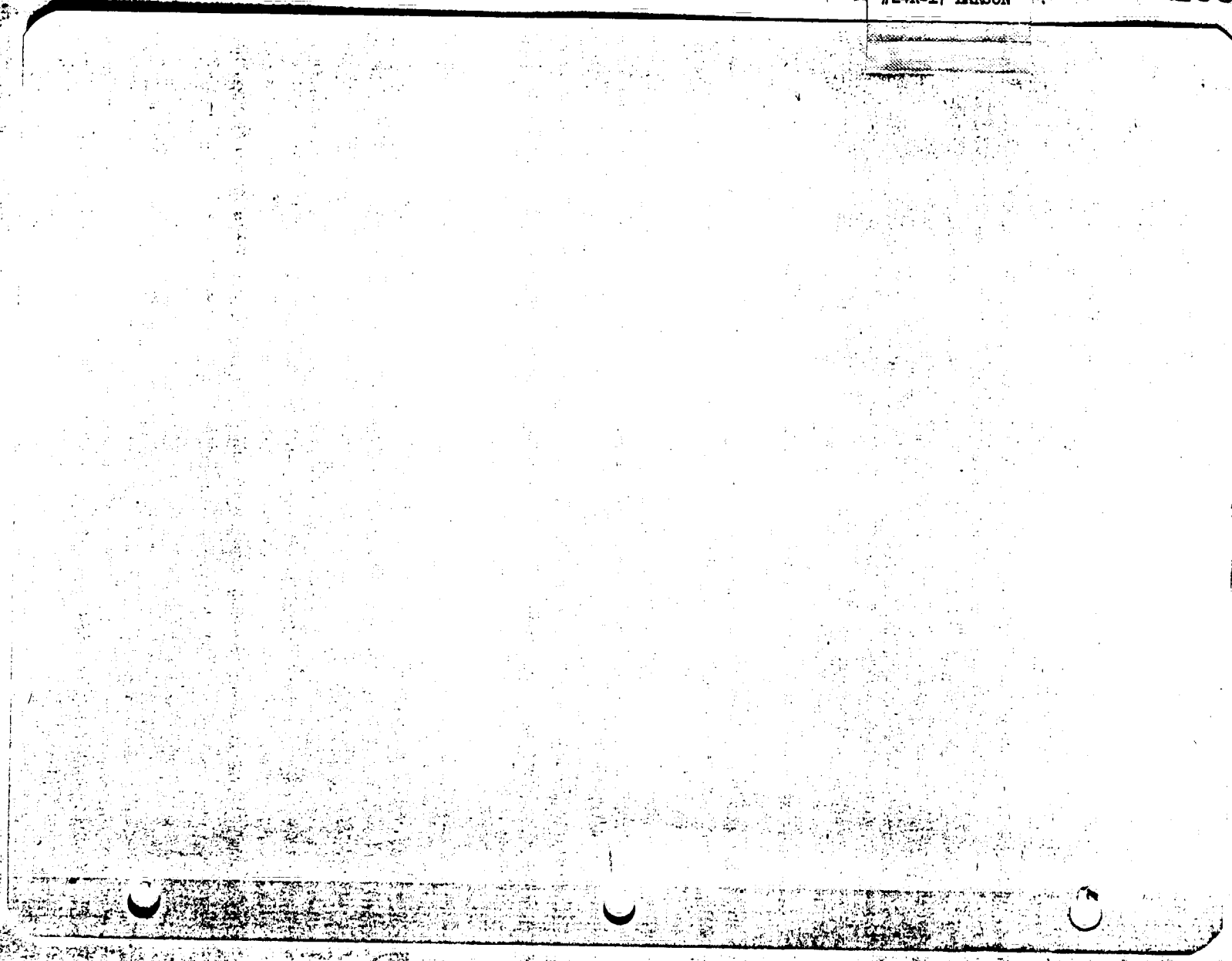

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THE COUNCIL FOR TOBACCO RESEARCH – U.S.A., INC.

P-1

January 23, 1973

Grant Application No. 24R-17

To: The committee comprising Drs. Bing, Cattell and Sommers

Subject: Paul S. Larson, Ph.D., Medical College of Virginia, Richmond
Renewal Application No. 24R-17
"Abstracting and classifying the literature on the biological effects of tobacco and preparation of manuscript for Supplement III to Tobacco (1961)."

History

This is the regular annual renewal of a grant that has been funded since 1954.

Application No. 24R-17 requests $49,795.00. The previous grant was $33,683.00. The increase is due in part to the usual "cost of living" increments, but mainly to inclusion for the first time of one-third salary for Dr. Larson. He retired as Chairman of the Department of Pharmacology in 1972.

Document Submitted (attached)

Application dated January 16, 1973.

Comment

Item No. 6 on the face page of the application summarizes current status. The plan is for termination of the project with publication of Supplement III in 1974.

F.W.N.

FWN/vr
Encl.

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MISCELLANEOUS

No. 243-17
Act: 11/1/54
Rec: 8/1/57 and annually each year since on July 1.

THE COUNCIL FOR TOBACCO RESEARCH–U.S.A., INC.

110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

NOTIFY:
Dr. Bing
Dr. Cattell
Dr. Somers

RECEIVED
JAN 22 1973
RESULTS

Application For Renewal of Research Grant

(Use extra pages as needed)

First Renewal ☐ Second Renewal ☐

Date: Jan. 16, 1973

1. Principal Investigator (give title and degrees): Paul S. Larson, Ph.D., Haag Professor of Pharmacology.

2. Institution & address: Medical College of Virginia, Health Sciences Division, Virginia Commonwealth University, Richmond, Virginia.

3. Department(s) where research will be done or collaboration provided: Department of Pharmacology.

4. Short title of study: Abstracting and classifying the literature on the biological effects of tobacco and preparation of manuscript for Supplement III to Tobacco (1961).

5. Proposed renewal date: July 1, 1973.

6. How results to date have changed earlier specific research aims:

Our goal remains that of completion of manuscript for Supplement III by June 1974. Manuscript drafts of Chapters 1 through 14, plus 16, plus most of Chapter 15, have been prepared. Working abstracts for Supplement III now number about 2750 with no let-up in accumulation.

7. How results to date have changed earlier working hypothesis: No change.

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8. Any additional facilities now required? Describe briefly: None

9. Any changes in personnel? Append biographical sketches of new key professional personnel: None

10. Append outline of experimental protocol for ensuing year. No change.

11. List publications or papers in press resulting from this or closely related work. (append reprints or manuscripts not previously sent).

Tobacco:Experimental and Clinical Studies (1961)

Supplement I (1968)

Supplement II (1971)

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12. Summary progress report (append in standard form as separate document, unless recently submitted). See 6 - above.

Source: https://www.industrydocuments.ucsf.edu/docs/rjfl0000

13. Budget for the coming year:

A. Salaries (give names or state "to be recruited")	% time	Amount
Professional (give % time of investigator(s) even if no salary requested)		
Paul S. Larson, Ph.D.	33	$11 500
Herbert Silvette, Ph.D.	100	21,500
Technical		
Secretary	100	5,640
Benefits		3,864
	Sub-Total for A	$42,504
B. Consumable supplies (by major categories)		
Supplies		800
	Sub-Total for B	800
C. Other expenses (itemize)		
None		
	Sub-Total for C	
	Running Total of A + B + C	$43,300
D. Permanent equipment (itemize)		
None		
	Sub-Total for D	
E. Indirect costs (15% of A+B+C)	E	$ 6,495
	Total request	$49,795

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Source: https://www.industrydocumentslibrary.ucsf.edu/docs/ytyb0000

14. Other sources of financial support:

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Preparation of a scientific resume' of published findings under the AMA-ERF Project for Research on Tobacco and Health	AMA-ERF	$56,645	10/1/72 - 9/30/75

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name Paul S. Larson

Signature Paul S. Larson Date 1/16/73

Telephone 703 770-5543
Area Code Number Extension

Checks payable to

Mr. Raymond T. Holmes

Mailing address for checks

Vice President for Finance
Virginia Commonwealth University
1200 East Broad Street
Richmond, Va. 23298

Responsible officer of institution

Typed Name M. Pinson Neal, Jr., M. D.

Title Ass't. Vice President, Health Science Div.

Signature M. Pinson Neal, Jr. Date 1/16/73

Telephone 703 770-5150
Area Code Number Extension

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